STUDIES ON THE MICROFLORA OF FARM ENVIRONMENTS ASSOCIATED WITH FARMER'S LUNG DISEASE

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SUMMARY

A study of the microflora of various types of farm buildings and products was made in Ayrshire and East Lothian and also of farm products in Orkney, with respect to the prevalance of farmer's lung disease in these three regions of Scotland. It was established that there was a correlation between the prevalence of farmer's lung and the state of preservation of farm products such as hay and grain, measured by the numbers of thermophilic organisms isolated both from the farm products and farm buildings. A lower state of preservation of materials in Orkney and Ayrshire, compared with East Lothian correlated with disease prevalences of 86, 86 and 23 per 1000 of the farming population, respectively. The hazards implicated in causing disease symptoms were hay, straw and grain.

Air-sampling of one dairy byre in the East of Scotland was carried out and it was established that high concentrations of propagules of fungi and actinomycetes were recovered from such a building, particularly after unbaling of hay. Figures of up to 7.31×10^6 fungal propagules per cubic metre of air were recorded by using the Burkard volumetric spore trap after unbaling of hay. Air-sampling by the Andersen Sampler was more accurate in quantitative and qualititative terms than that by either the Burkard volumetric spore trap or the gravity Petri-dish technique in isolating fungi and actinomycetes.

Air-sampling of farm buildings on Ayrshire dairy farms by using the Andersen Sampler established that there was, in general, a very high concentration of organisms in the air of such buildings after unbaling of hay in dairy byres and bruising of grain in bruising-sheds. There was a correlation between high numbers of thermophilic organisms, particularly the actinomycete, Micropolyspora faeni, both from the air of farm buildings and from products used in these buildings and the presence of farmer's lung disease on the dairy farms studied. In general, farms with no evidence of farmer's lung disease in the six years previous to the study realised considerably lower numbers of colonies of M. faeni both from farm buildings and from materials such as hay, grain and dust.

Only 43% of sera from individuals with clinical symptoms of farmer's lung were positive to standard farmer's lung hay (F.L.H.) antigen in the initial survey of Ayrshire, Orkney and East Lothian. Antigenic extracts of material from Ayrshire dairy farms and from fungi and actinomycetes isolated from these farm buildings and products were tested against sera from personnel on the farms surveyed. Extracts of hay and grain from farmer's lung disease (FLD) farms were more reactive than those from non-FLD farms but did not correlate directly with reactivity to standard F.L.H. antigen. Fifty-two percent of personnel from FLD farms were serologically positive to F.L.H. or when tested against extracts of M. faeni and 54% of personnel from non-FLD farms were positive to such extracts, but of the former group, 67% were also symptomatic. There was a similar pattern of sensitisation to F.L.H. and to M. faeni on both FLD and non-FLD farms which did not correlate with disease symptoms or the concentration of M. faeni on the farms concerned. Extracts of organisms other than M. faeni did not prove to be significantly serologically reactive. Neither F.L.H. antigen nor extracts of M. faeni appeared to be diagnostically significant but did indicate a population at high risk to farmer's lung disease by acting as a measure of the state of preservation of vegetable products. Some future refinement of serological testing to differentiate between diseased and non-diseased sensitised individuals was postulated.

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Introduction

The first clinical descripton of farmer's lung disease was given by Campbell (1932), and was associated with farm-workers handling mouldy hay. It has since been described by investigators in Britain and the United States of America, including Fuller (1953), Dickie and Rankin (1958), Frank (1958), Staines and Formarr (1961) Rankin et al.(1962), Bishop et al.(1963) Williams (1963), Emanuel et al.(1964), Wenzel et al (1964), Rankin et al. (1965,1967), Hapke et al. (1968), Seal et al. (1968), Barrowcliff and Arblaster (1968) and Grant et al. (1972) and has been reviewed by Pepys (1969), Stretton (1972) and Blyth (1973).

Farmer's lung disease is now considered to be a form of extrinsic allergic alveolitis. It is attributable neither to direct physical damage to the lung tissue nor to invasion by a pathogen but to a form of allergic response. The material which initiates this response within the alveoli is contained in dust emanating from hay or other vegetable products. There are two main types of allergic response to such material. One is shown by sufferers from asthma or rhinitis, who are described as atopic and give an immediate type I reaction when in contact with allergen due to the interaction of the allergen with reagin or IgE antibodies and the production of vasoactive substances such as histamine. The clinical effects are manifested as 'hay fever' or an asthmatic attack resulting from the release of secretions from the upper or lower respiratory tract, respectively. The majority of the population are non-atopic individuals and are characterised by having a lower level of IgE antibodies than atopic individuals and also by the fact that a large proportion of this group show antigenic reactivity in the form of a type III, Arthus reaction (Gell and Coombs, 1968). Circulating antibodies of the IgA, IgG and IgM type, reacting with antigen in the small blood vessels of the alveoli to form precipitates several hours after the allergen has been inhaled, are believed to cause the initial symptoms of a disease such

as farmer's lung.

Initial symptoms are characterised by sensations of shortness of breath or suffocation, accompanied by feverishness, chills and malaise, akin to symptoms of influenza, and a dry cough. Subsequent exposure to the dust hazard may lead to breathlessness and a dry cough but may also result in the production of a purulent sputum. Mottling of the central and lower portions of the lung can be apparent at this stage, on x-ray examination. The presence of granulomata in the lung during this acute stage is also characteristic. There is a reduction in pulmonary function, measured by forced vital capacity, carbon monoxide transfer factor and partial pressure of oxygen in the blood. Recovery from this stage of the disease can take place if the patient no longer comes in contact with the antigen causing disease symptoms. The permanent, chronic stage of the disease may arise insidiously i.e. after repeated exposure to small quantities of antigen or may arise after one or more acute attacks. In the chronic stage, symptoms of cough and shortness of breath cannot be removed by lack of contact with the antigen. Fibrosis of lung tissue interstitially and around granulomata, arterioles and bronchioles causes permanent lung damage but clinical symptoms at this stage of the disease are variable. The disease is then mildly or severely disabling and can prove to be fatal.

As the disease can cause such disability and force workers to leave their employment, it is registered as an occupational disease and is defined as "pulmonary disease due to the inhalation of the dust of mouldy hay or of other mouldy vegetable produce, and characterised by symptoms and signs attributable to a reaction in the peripheral part of the bronchopulmonary system and giving rise to a defect in gas exchange". (Ministry of Pensions and National Insurance, 1964).

As the response to allergen is, by definition of the disease, believed to be initiated in the alveoli, particles of dimensions capable of penetrating to these regions are believed to be involved in the disease. Hatch and Gross

(1964), Austwick (1966), Druett (1967) and Gregory (1973) have described the structure of the respiratory system and reviewed information on the retention of particles by various regions of the respiratory tracts. Large particles are retained by the nasal hairs and,of those which pass this initial barrier, the majority with diameters greater than 20μ are caught in the mucus and ciliated epithelial cells of the nasal cavity. These are then transported to the pharynx and swallowed. The largest proportion of particles between approximately 4μ and 20μ diameter are retained by the ciliated epithelium of the nose, trachea, bronchi and bronchioles. Those deposited in regions from the trachea downwards, are transported upwards to the hypopharynx and swallowed. The majority of particles between 0.5μ and 4μ diameter are retained in the alveoli. Deposition of particles in the different regions of the respiratory tract is dependent on their size, which also determines terminal velocity and rate of deposition from a current of moving air. The rate at which air moves in the respiratory tract decreases between the nasal cavity and the alveoli from 150 cm/sec to 1cm/sec. and allows rapid deposition of larger particles in the upper regions of the tract. As this sedimentation rate decreases with decreasing velocity of air and smaller particle size, more than fifty percent of particles of below 1μ are exhaled. The percentage exhaled reaches a maximum at approximately 0.5μ diameter. Below 0.4 μ diameter, due to Brownian motion, an increasing proportion of particles are in contact with the surface of the alveolar cells and are retained by this lung tissue. The majority of particles retained by the alveoli and those which would be considered most likely to initiate an allergic response are below 4μ. in diameter but up to 20% of particles of 5μ diameter and negligible numbers of 10μ diameter (Lidwell, 1970) can be retained by the alveoli.

Other forms of extrinsic allergic alveolitis include maple-bark stripper's lung (Emanual et al.,1966), bird-fancier's lung (Reed et al.,1965), mushroom-

worker's lung (Sakula, 1967), Bagassosis (Salvaggio et al., 1966) Sequoisis (Cohen et al., 1967) and malt-worker's lung (Riddle et al., 1968) The identification of the cited causative agents of these diseases and of farmer's lung disease is not merely a function of their ability to penetrate to the peripheral regions of the lung but is dependent on their capacity to provoke antibody formation. By exploiting serological techniques and also by inhalation tests probable causative agents have been identified. The sequence of events involved in establishing which organisms are considered to be responsible for causing farmer's lung disease is described below.

Antigenic extracts of mouldy hay were found to form preciptin arcs against sera from 60 of 71 patients with farmer's lung disease, using the double diffusion technique of Ouchterlony 1953 (Pepys et al., 1962). Cross-absorption of these sera with fungal extracts did not inhibit reactions to extracts of mouldy hay and these extracts were said to contain farmer's lung hay (F.L.H.) antigen. In a later paper, Pepys and Jenkins (1963) stated that extracts of the thermophilic actinomycete, Thermopolyspora polyspora (later renamed Micropolyspora faeni: Cross et al., 1968) contained many antigenic components in common with F.L.H. and was, therefore, considered to be involved in causing the disease. Arcs of preciptation were formed in characteristic A, B and C regions during immunoelectrophoresis, which involves the separation of antigenic components according to their net charge and movement through an electrical field. The number of regions in which arcs were formed (0 to 3) was related, though not absolutely to the clinical severity of disease (Pepys and Jenkins 1965). This grading was also related to the smaller number of reactions of these sera to extracts of another thermophilic actinomycete, Thermoactinomyces vulgaris and to extracts of a Mucor sp. and Aspergillus fumigatus. Extracts of T.vulgaris were positive to 52% of sera from patients with clinical symptoms of farmer's lung disease and yielded preciptin arcs only in the C region of immunoelectrophoresis.

In the same set of sera,87% from patients with clinical symptoms reacted to extracts of M. faeni. No reactivity of these organisms was found in sera from urban dwellers but 18% of apparently healthy farmers were serologically positive to extracts of M. faeni, although less strongly than diseased individuals. Other investigators have confirmed this correlation between disease symptoms and serological reactivity to extracts of M.faeni and T. vulgaris (Kobayashi et al (1963), Emanuel et al. (1964), Davies and Yull (1966), Rankin et al.(1967) Wenzel et al.(1968), Hapke et al.(1968) Fletcher,Rondle and Murray (1970). Hapke et al.(1968) established that although 100% of sera from patients in the acute stage of the disease were serologically positive to extracts of M. faeni, only 53% from those in the chronic stage of the disease were positive and questioned the value of this serological test in diagnosis of farmer's lung disease. Hapke et al. (1968) also demonstrated that other, unidentified antigens, present in a range of hay samples reacted in serological tests against a proportion of sera which were negative to M. faeni and they postulated that antibodies to these antigens persisted for a longer time than those of M. faeni in chronically diseased patients.

Evidence which supports the involvement of F.L.H. antigen and of M.faeni in the aetiology of farmer's lung disease is the provocation of symptoms after inhalation of extracts from mouldy hay and from M. faeni. Twelve of fifteen farmer's lung patients produced symptoms of a rise in temperature and decreased pulmonary function several hours after inhalation of aerosols containing aqueous extracts of mouldy hay (Williams, 1963). Barbee et al., (1965) and Pepys and Jenkins (1965) using extracts of M. faeni provoked similar reactions and Pepys and Jenkins (1965) also provoked reactions to extracts of T. vulgaris, but to a lesser extent. Extracts of good hay did not provoke symptoms, nor did those of fungi, including Aspergillus spp., Mucor spp. and Penicillium spp.but in one case disease symptoms resulted

from an extract of the coarse fraction of mouldy hay. As both M. faeni and T. vulgaris are thermophilic actinomycetes, they would be of the dimensions to predominate in the fine but not the coarse fraction of such dust.

Refinements of antigen extraction from mouldy hay and from M. faeni have been made by several workers. To obtain farmer's lung hay (F.L.H.) antigen, extraction of hay samples by Pepys et al.,1962,1963 was achieved by the use of carbol saline (Coca's solution) after defatting in acetone. Freeze-thaw extracts of M. faeni grown on agar media and of M. faeni grown on cellophane above agar media, when added to test sera, inhibited reactions to F.L.H. antigen indicating that the organism could produce antigens without growth on a natural substrate. Kobayashi et al.(1963),Pepys and Jenkins (1965) and Laberge and Strahmann(1966 a and b) extracted mouldy hay and M. faeni with trichloracetic acid (T.C.A.). Pepys and Jenkins (1965) established that the T.C.A. soluble fraction was predominantly that which reacted in the C region during immunoelectrophoresis. Laberge and Stahmann(1966 a and b), however, demonstrated that the T.C.A. soluble fraction produced precipitin arcs in the A,B and C regions during immunoelectrophoresis but these arcs differed from those of Pepys and Jenkins (1965). Provocation of symptoms by the T.C.A. soluble fraction was established by Barbee et al. (1965) in six farmer's lung disease patients but not in ten controls who exhibited only slight rises in temperature and in white cell counts. Pepys (1969) claimed that the A,B and C arcs of Laberge and Stahmann(1966 a and b) were not representative of the A and B arcs of Pepys and Jenkins (1965), which resulted from antigens precipitated by T.C.A. and which were primarily protein in constitution. The T.C.A. soluble fraction of mouldy hay (Laberge and Strahmann,1966 a) proved to consist of glycoproteins and the linkages between the peptides and carbohydrates were sufficiently strong to withstand T.C.A. extraction, alcohol fractionation and chromatography on DEAE-cellulose ion-exchange columns. Trichloracetic acid

soluble extracts of M. faeni (Laberge and Stahmann, 1966 b) were shown to have the same immunoelectrophoretic and biochemical properties as those from mouldy hay and were chiefly polysaccharides containing galactose, arabinose and glucosamine, together with a glycopeptide which was apparently of cell wall origin. Similarly, Rankin et al. (1967) demonstrated that the polysaccharide fraction of T.C.A. soluble extracts of M. faeni were antigenic. Kobayashi et al. (1963) claimed that T.C.A. soluble antigens were specific in differentiating between sera from patients with farmer's lung disease and those without. Wenzel et al. (1968) used 0.2 M pyridine acetate buffer to extract antigens which consisted predominantly of those which precipitated in the C region of Pepys and Jenkins (1965) and obtained positive serological reactions in double-diffusion of these extracts against sera from all of 25 acute cases of farmer's lung disease tested. Utilising the double-diffusion technique of Ouchterlony (1953), Fletcher et al., 1970, obtained 29 precipitin arcs against a combination of sera from farmer's lung disease patients. The antigens were recovered by concentrating broth from liquid cultures of M. faeni and were, therefore, extracellular. These workers established that the numbers of precipitin arcs produced by sera of individuals with severe clinical symptoms of farmer's lung disease were higher than from those with less severe symptoms, but there was no obvious difference between severe and mild forms of the disease in the distribution of precipitin arcs. They obtained positive results in 75% of sera from farmer's lung disease patients and from 20% of individuals exposed to the same environment. There was no difference in the distribution of precipitin arcs between the two groups of positive sera but most of the precipitin positive group without farmers' lung symptoms had some other form of respiratory disease.

Immunoelectrophoresis is less sensitive than double-diffusion in determining weakly reactive sera but Jameson (1968), using immunoosmophoresis produced preciptin arcs against sera which had previously been negative in double diffusio

He also came to the conclusion that strong reactions to concentrated broth extracts from M.faeni cultures and also reactivity to a dialysate of broth medium, were reliable indications of farmer's lung disease. In a subsequent paper, Jameson(1969), concluded that mycelial antigens were less reactive than those from broth concentrates and differentiated between two groups of farmer's lung patients on the basis of reactivity to an antigenic component from concentrated broths. He suggested that symptoms in one of these groups of patients were not related to M.faeni. Edwards(1971)utilised a double-dialysis method of recovering antigenic components of M. faeni from culture media free from the macromolecules of the medium itself. He established that a larger quantity and a wider range of antigens were recovered by this method than by conventional liquid culture. Recently, Hollingdale (1973) concluded that there were two groups of mycelial antigens in M. faeni, one of which contained three glycoprotein antigens, sensitive to sodium periodate which were diagnostic of farmer's lung disease. Gray et al.(1969) and Parrat and Peel (1972) described the use of the fluorescent antibody technique in evaluating the presence of antibody in sera and in Grant et al.,1972, applications of this technique by Parrat and Peel realised positive results in 16 of 18 symptomatic indivuals and negative results in 6 of 7 asymptomatic persons. The latex agglutination test (Murray et al.,1967) and the complement fixation test (Murray,1967) have also proved to be of value in assessing serological reactivity of farmer's lung patients.

Antibodies to F.L.H. antigen have also been found in both healthy and diseased cattle (Jenkins and Pepys,1965: Pirie et al.,1972). In some animals serological positivity was related to respiratory disease, formerly described as fog-fever, a term which refers to a variety of respiratory disorders in cattle. Pirie et al.,1971, described typical allergic alveolitis in a cow which was serologically positive to M. faeni and described the disease as bovine extrinsic allergic alveolitis. Pirie et al.,1972, demonstrated that

a larger proportion of cattle from herds associated with cases of farmer's lung disease were serologically positive to M. faeni than that of control herds.

In a series of papers the airborne microflora of hay and grain was examined with respect to F.L.H. antigen production and in relation to human and animal respiratory disease (Gregory and Lacey, 1963 a and b; Gregory et al., 1963; Gregory et al., 1964; Festenstein et al., 1965; Lacey, 1968). Gregory and Lacey (1963 a)using a wind tunnel in conjunction with a cascade impactor (May, 1945) and Andersen Sampler (Andersen, 1958) established that mouldy hays associated with cases of farmer's lung disease yielded larger numbers of spores per gram dry weight than other mouldy hays or good hay samples. 'Farmer's lung' hays were characterised by having larger numbers of spores of Humicola lanuginosa and Aspergillus fumigatus and particularly large concentrations of actinomycete spores. Lacey, 1968, evaluated the microbiology of mouldy hay, grain and straw associated with bovine respiratory disease and found that from 29 of the 41 farms surveyed samples characteristic of 'farmer's lung' type fodder were collected. Values of 39.6 to 817.9 actinomycete spores/g. dry weight and 1.0 to 92.6 fungal spores/g. dry weight were obtained together with large numbers of the thermophilic actinomycetes M. faeni and T. vulgaris.

Farmer's lung type hay is that which shows evidence of having self-heated considerably due to an initially high moisture content when stored. Gregory et al., 1963, established that hay stored at 16% moisture content showed little evidence of self-heating, hay stored at 25% moisture content heated to 45°C but storage at 40% moisture content could result in temperatures of 60°C to 65°C. The production of F.L.H. antigen was associated with hay which was baled at more than 30% moisture content and which had a higher pH. (7.0) than other hays. The antigens formed were predominantly those which were common to M. faeni and to a lesser extent, T. vulgaris. In an experimental situation,

Festenstein et al., 1965, stored hay and grain of different moisture contents in aerated Dewar flasks and established that the production of antigens which gave reactivity in A, B and C regions during immunoelectrophoresis was related to the growth of the thermophilic actinomycete, M. faeni. Samples which were stored at between 29% and 34% moisture content reached temperatures of between 33°C and 55°C and yielded various concentrations of F.L.H. antigen, correlating with the numbers of colonies of M. faeni isolated from the samples. Hay stored at 40% moisture content heated to approximately 65°C and produced relatively high concentrations of F.L.H. antigen and numerous spores of M. faeni. Original moisture contents above 47% , however, yielded progressively less antigen. Characteristic microflorae were associated with different ranges of moisture content and species of Aspergilli and thermophilic actinomycetes could be related to original moisture content, as was established for baled hay.

Investigations therefore indicated that there was a source of antigen associated with the disease in farm products which had self-heated. The use of the wind-tunnel to remove spores from these products demonstrated that large concentrations of spores could be airborne but did not relate these concentrations to normal working conditions. Measurements of spore concentrations in farm buildings were made by Baruah (1961) and Lacey and Lacey (1964). Baruah (1961) quoted figures of between 95,000 and 16 million fungal spores per cubic metre of air, obtained by a volumetric spore trap placed in a cowshed under normal working conditions. Lacey and Lacey (1964) recovered up to 1600 million spores/m from a cascade impactor after hay had been shaken in a cowshed. Ninety-eight percent of these spores were of actinomycetes. Although a large number of species of actinomycetes can be recovered on agar media (Corbaz, et al., 1963) the only organisms of this group which have been proved to be consistently antigenic with respect to farmers lung disease were M. faeni and T. vulgaris (Jenkins, 1964). Any antigenicity in extracts from cultures of fungal species has also proved to be inconsistent with disease symptoms.

Reported figures for the prevalence of farmer's lung disease in East Anglia, Southwest England and Wales are 11.5 per 100,000, 73.1 per 100,000 and 193.1 per 100,000 respectively (Staines and Forman, 1961). The only figure available for Scotland was that of Boyd, 1971, with a prevalence of 110 per 100,000 for Caithness. For that reason, a survey of the farming populations in Orkney, Ayrshire and East Lothian was made (Grant et al., 1972) to compare three regions which had different climates and agricultural practice with respect to farmer's lung disease. The aim of the present thesis was to relate the microbiology of the air of farm buildings and of vegetable products used in these buildings to the clinical findings of the survey. One farm building in the east of Scotland in a location comparable with the climate in East Lothian, was used to evaluate the merits of different air-sampling techniques and to estimate fluctuations in concentrations of microorganisms under normal working conditions. In Ayrshire, where the prevalence of disease proved to be high (86 per 1000), the microbiology of selected farm environments was related to clinical diagnosis of farmer's lung disease and serological reactivity of individuals on these farms.

SECTION I

THE MICROBIOLOGY OF A DAIRY BYRE: TO ESTABLISH THE RELATIONSHIP BETWEEN ACTIVITIES OF ANIMAL HUSBANDRY AND THE CONCENTRATION OF AIRBORNE PROPAGULES OF FUNGI AND ACTINOMYCETES.

The microbiology of a dairy byre: to establish the relationship between activities of animal husbandry and the concentration of airborne propagules of fungi and actinomycetes.

Materials and Methods

The microbiology of a modern, two-span dairy byre, housing 64 dairy cattle in Fife, was studied to estimate the variation in concentrations of propagules of fungi and actinomycetes during normal working conditions. It is general practice to house the cattle from the beginning of November to the beginning of the following May but this is dependent on the length of a particular Winter season.

Sampling of the air in the byre was carried out during the third or fourth week of March from 1969 to 1972. The animals had then been indoors for approximately five months, the baled hay being used for fodder had been stored for nine months and the baled straw for bedding, for six months. Barley, which had been stored for six months was bruised and mixed with commercially available cattle concentrate for feeding. Storage times are approximate, depending on the time of harvesting. Maize draff, a by-product of the brewing industry , was also fed to the animals.

The routine for a normal working day with approximate times, is shown in Figure 1.

1) Air-sampling by the Burkard, Seven-day, Volumetric Spore Trap

A Burkard, seven-day, volumetric spore trap (Figure 2) was run at an air-flow of 10 litres per minute in the byre, described above (Figures 3 and 4). The orifice of the trap was 2.5 metres from ground level and the total volume of the byre was 1,341 cubic metres (length 17.06m., breadth 16.14m. and average height 4.87m.). Trapping took place on six consecutive days during the third or fourth weeks of March 1969, 1970, 1971 and 1972.

Approximate Time	Activities of Animal Husbandry
05.15	Milking of dairy cattle started
05.30 - 05.40	Draff fed to animals
06.20 - 06.30	Bruised barley and concentrate fed
07.20 - 07.30	Cattle foddered with hay: Milking completed
09.00 - 09.15	Byre mucked with mechanical mucker
09.15 - 10.15	Turnips fed to cattle
10.15 - 10.30	Animals bedded with barley straw
10.30 - 10.40	Byre walks washed
15.15	Milking started
15.30 - 15.40	Bruised barley and concentrate fed
17.10.- 17.20	Cattle foddered with hay
17.10 - 17.20	Animals bedded with barley straw
17.25 - 17.30	Byre walks washed

Figure 1: The daily working schedule in the dairy byre with approximate times of activities of animal husbandry.

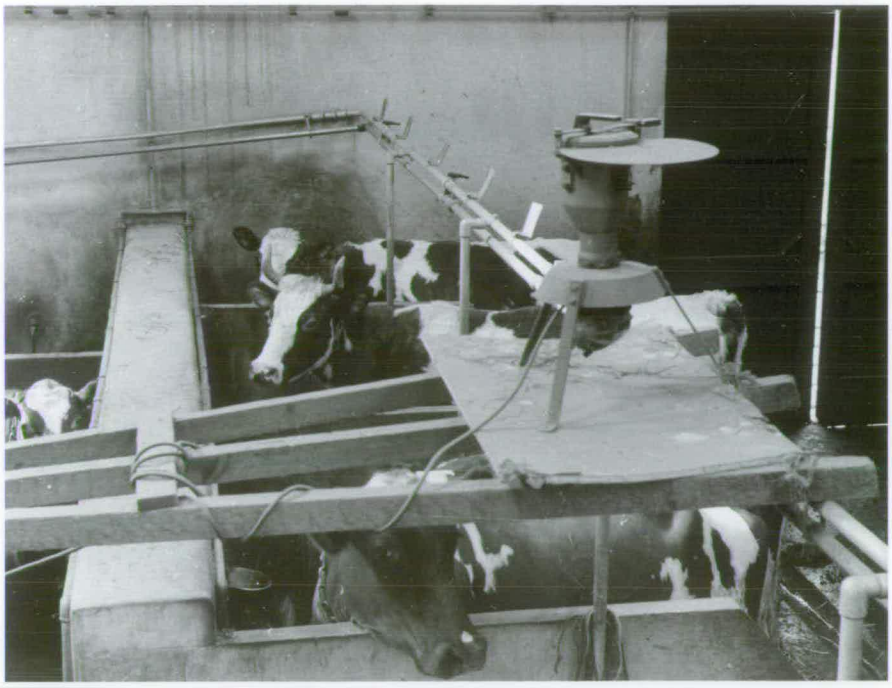


Figure 2 : Burkard seven-day volumetric spore trap in position in a dairy byre.



Figure 3 : Burkard seven day volumetric spore trap with orifice 2.5m. above ground level in a dairy byre.

⊕:position of spore trap

□:position of Andersen sampler

S :cattle stalls

W :walk

P :passage-way

═:doorways

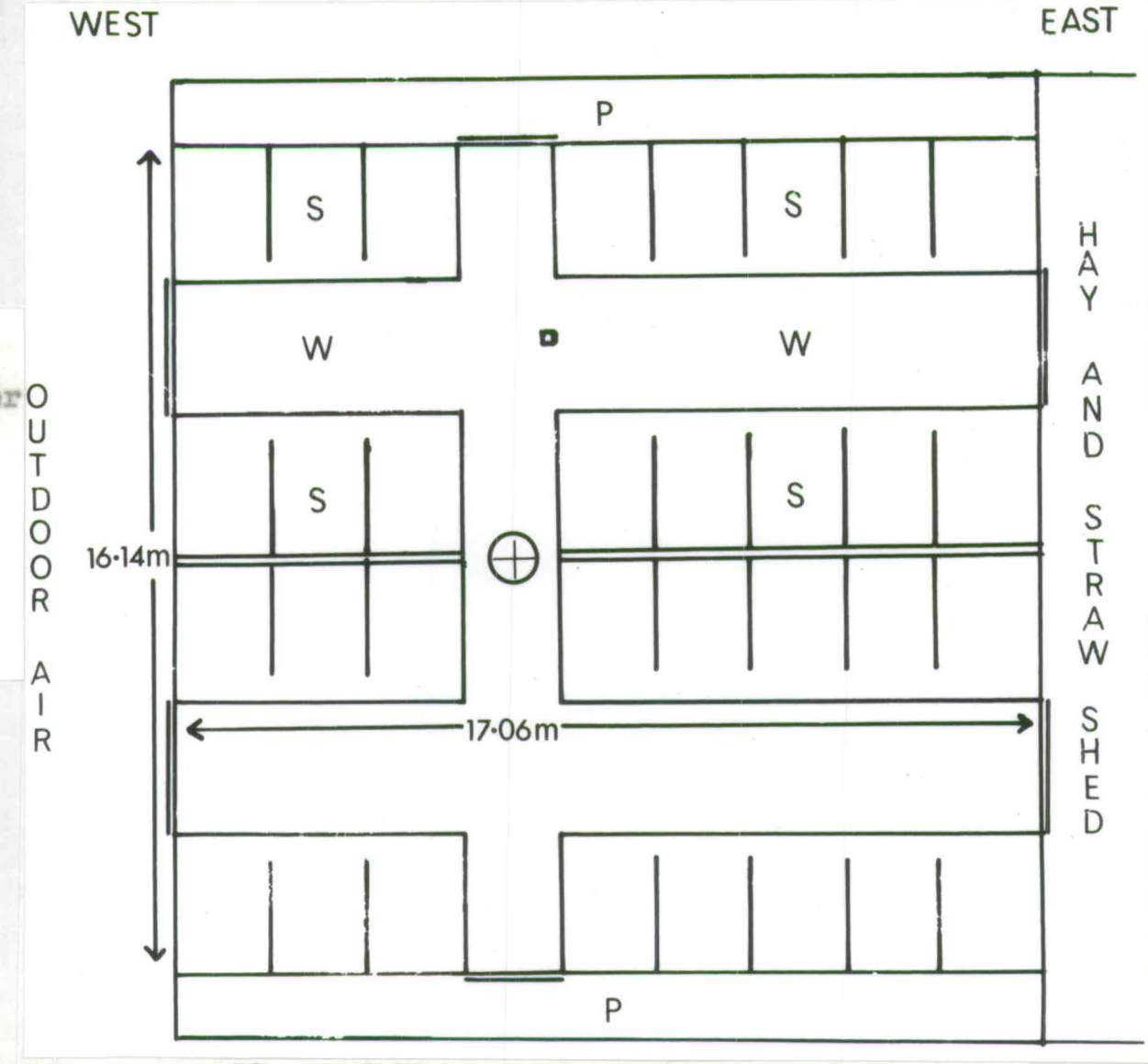


Figure 4:Diagram of the two-span dairy byre.

A cellophane strip, smeared on both sides with vaseline, was applied, as directed by the makers, (Burkard Manufacturing Co. Ltd.) to the metal drum inside the trap. Tapes were loaded into the trap at times during the day when the particle concentration in the byre was thought to be low, to minimise contamination of the tape. The drum rotated at the rate of 2mm. per hour and one complete rotation took 7 days. The cellophane strip was removed after the requisite time and cut into sections equivalent to 24-hour intervals, using the calibrated ruler provided with the trap. The sections were mounted in gelvatol on 76mm x 25mm glass slides under No. 1 22mm x 64 mm cover-glasses.

The numbers of propagules of fungi and actinomycetes/bacteria on the spore trap traces were counted at x 400 magnification using a Gillett and Sibert Conference microscope fitted with a 0.5cm x 0.5cm eyepiece graticule divided into 100 squares. The numbers of fungal propagules per cubic metre of air was calculated for every 3 min. 45 sec. (1/16th hr.) over one 24-hour period, from 0.00 hr. to 24.00 hr. on March 16th, 1970.

Each length of trace equivalent to 1 hour, i.e. 2mm, was divided into 16 parts and the numbers of propagules per cubic metre was calculated, as follows:

x = Number of propagules per 1/16th hour on 1 grid-width of trace.

16.x = Number of propagules per hour on 1 grid-width of trace.

Width of trace = 115.5 x Width of grid.

Therefore, Number of propagules per hour = $x.16.115.5$

Number of propagules per min. = $\frac{x.16.115.5}{60}$

The trap runs at an air-flow of 10l/min.

Therefore, Number of propagules per litre = $\frac{x.16.115.5}{60.10}$

Number of propagules per cubic metre = $\frac{x.16.115.5.1000}{60.10}$

= $x.3080$

The numbers of propagules at peak concentration periods were calculated from data obtained between the afternoon of March 15th and the morning of March 21st, 1970.

After 3 morning (on the 16th, 17th and 21st) and 3 evening (on the 16th, 17th and 20th) peak concentrations of propagules in March 1970, the total numbers and the numbers of different types of fungal propagules were counted at alternate 15 minute intervals for up to 5 hours 45 mins. after morning peak concentrations and for up to 6 hours 15 mins. after evening peak concentrations. Counts were also made at 8 hours, 10 hours and 12 hours after evening peak concnetrations. Peak density was taken as the 1/16th hour interval at which the highest number of fungal propagules was recorded and zero time was taken as the beginning of the next 1/16th hour interval. When multiple peaks were present, in the evening, the final major peak was used to give the reference peak concentration. The numbers of small refractile bodies of the dimensions of propagules of actinomycetes or bacteria were also counted at the times after morning and evening peak concentrations, described above.

2) Air-sampling by the Gravity Petri-dish Technique.

At specific times during the day the gravity Petri-dish technique described by Gregory, 1973, was employed. Two 9cm. plastic Petri-dishes containing agar media were exposed for 2 minutes on the platform supporting the spore trap (Figure 2). In this position, they were free from interference from both animals and people. One Petri-dish, subsequently incubated at 26°C., contained 2% malt agar (see Appendix A) and the other, subsequently incubated at 46°C., contained nutrient agar plus cycloheximide, to prevent fungal growth (see Appendix A).

The numbers of colonies of mesophilic micro-organisms, grown at 26°C. and of thermophilic actinomycetes grown at 46°C. were counted after 3 days incubation for 6 days in March 1969, 6 days in March 1970, and 3 days in March 1971.

The numbers of mesophilic fungal colonies were counted after 7 days, for the 6-day period in March 1970, and the 3-day period in March 1971. In 1971, fungal species were also identified.

Petri-dishes were exposed at the following times to estimate the effect of activities of animal husbandry on spore concentration in the byre:

Immediately after animals were fed with bruised barley and concentrate.

Immediately after and 10 minutes after unbaling of hay or unbaling of hay and straw.

Between morning and evening feeding times, in the early afternoon.

Approximately 4 hours after evening feeding and bedding of cattle.

In March 1971, the rate at which the numbers of fungal propagules decreased with time from peak concentration was estimated. On 3 consecutive days, 28th to 30th March, Petri-dishes containing 2% malt agar and incubated at 26°C. were exposed at 30 minute intervals for up to 3 hours after peak concentration. On 2 of these days, the 28th and 30th, Petri-dishes were exposed 4 hours, 5 hours and 6 hours after the evening peak concentrations, which was taken as 10 minutes after unbaling of hay for feeding and of straw for bedding the animals.

3) Air-sampling by the Andersen Sampler in Conjunction with the Gravity Petri-dish Technique.

On March 22nd 1971, an 8-stage Andersen Sampler (Andersen 1958 - See Appendix B) was used to estimate the numbers of viable propagules of fungi and actinomycetes in the atmosphere of the byre, in conjunction with the gravity Petri-dish technique. The orifice of the Sampler was 1m. from ground level. The Petri-dishes were exposed at approximately 0.8m. from ground level in the position marked on Figure 4. The Sampler was loaded with sets of the following plates:

2% malt agar, subsequently incubated at 26°C.

Nutrient agar plus cycloheximide, subsequently incubated at 46°C. (see Appendix A).

Glycerol asparaginate agar, subsequently incubated at 46° C. (see Appendix A).

at the following times in the evening:

17.40h. - 17.45h.: At peak particle concentration, 10 minutes after unbaling of hay and straw.

18.10h. - 18.15h.: 30 minutes after peak concentration

19.10h. - 19.15h.: 1hour 30 minutes after peak concentration

11.40h. - 11.45h.: 6 hours after peak concentration

The air-flow through the machine was 25l./m. for 10 seconds at the peak concentration, for 15 seconds at 30 minutes and 1 hour 30 minutes after peak concentration and for 30 seconds, 6 hours after peak concentration. The numbers of viable propagules per cubic metre were calculated, as described in Appendix B. Petri-dishes were exposed for 2 minutes at the times equivalent to those at which the Sampler was being run.

Results

1. Air-sampling by the Burkard Seven-Day Volumetric Spore Trap.

a) Macroscopic observations on spore traces

Photographs of spore traces obtained by using the Burkard Seven-day Volumetric Spore Trap for two days in March 1969 and two days in March 1972 are shown in Figure 5. The most dense bands of concentration of dust on both traces are at times of morning and evening foddering and bedding of animals (see Figure 1). Less dense banding patterns are seen during the day between feeding times but after evening foddering and bedding the bands become less discrete. There are slight fluctuations in the low level of dust concentration throughout the night until activity commences in the byre the following morning.

The 1969 spore trace (Figure 5a) contains a large concentration of white dust at times of foddering. The hay bales which were being used during this part of the study showed evidence of moulding and self-heating, resulting in the dense clouds of dust (Figure 6) which were released when the bales were opened and distributed. The white dust probably consists mainly of propagules of thermophilic actinomycetes (Gregory et.al., 1963).

In contrast to the 1969 trace, that for 1972 does not exhibit large concentrations of white dust but contains material of predominantly brown pigmentation (Figure 5b). Figure 7 demonstrates a portion of the 1972 trace, from 0.00 hr. to 24.00 hr. on one day. The least dense region of the trace is equivalent to the time immediately before morning activity was begun, and the concentration rose at morning feeding and foddering times. Banding patterns corresponding to times of activities such as mucking of the byre and bedding of the animals are evident during the day. After a very dense concentration of dust at evening foddering and bedding of the animals, the concentration decreased again.

During 1970 and 1971, traces similar to that for 1972 were obtained, i.e. they contained material of predominantly brown pigmentation. Results of detailed examination of mounted 24-hour traces for 6 days in 1970 are given below.

a

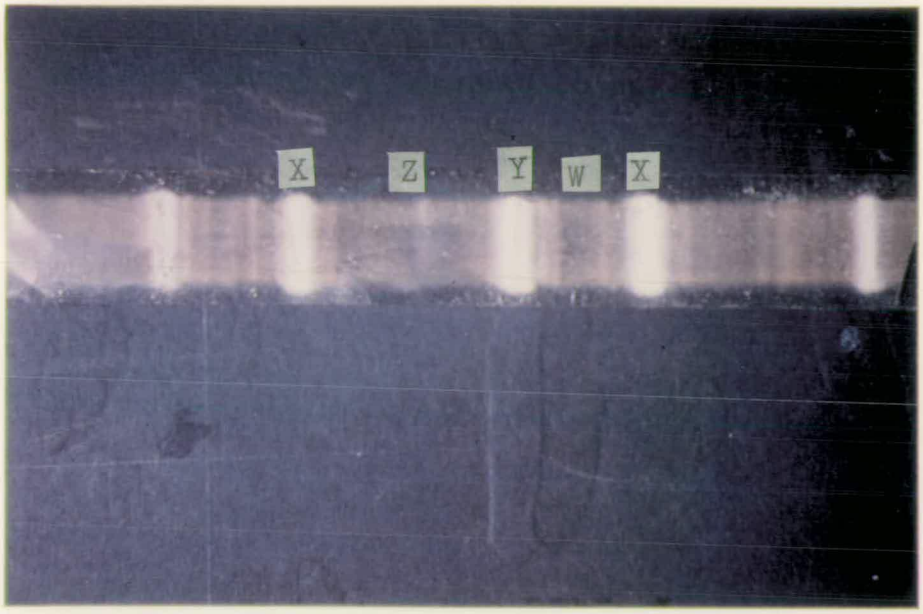


Figure 5: Spore traces covering approximately 48 hours, obtained by using the Burkard 7-day volumetric spore trap in a dairy byre in a) March, 1969 and b) March, 1972.

X-unbaling of hay in morning W-during day
Y-unbaling of hay in evening Z-during night
X-X approximately 24 hours.

b

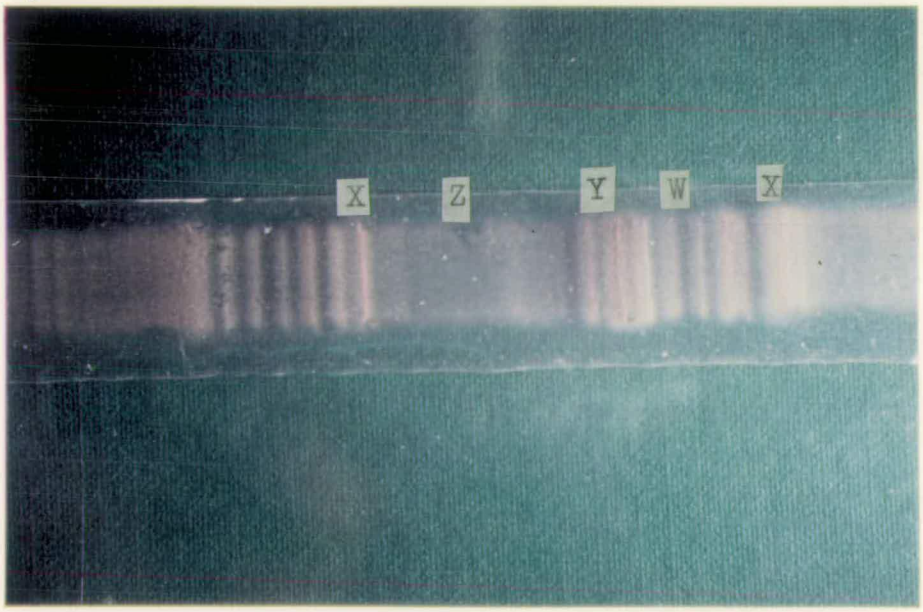




Figure 6 :'Spore cloud' arising after disturbance of a mouldy hay bale.

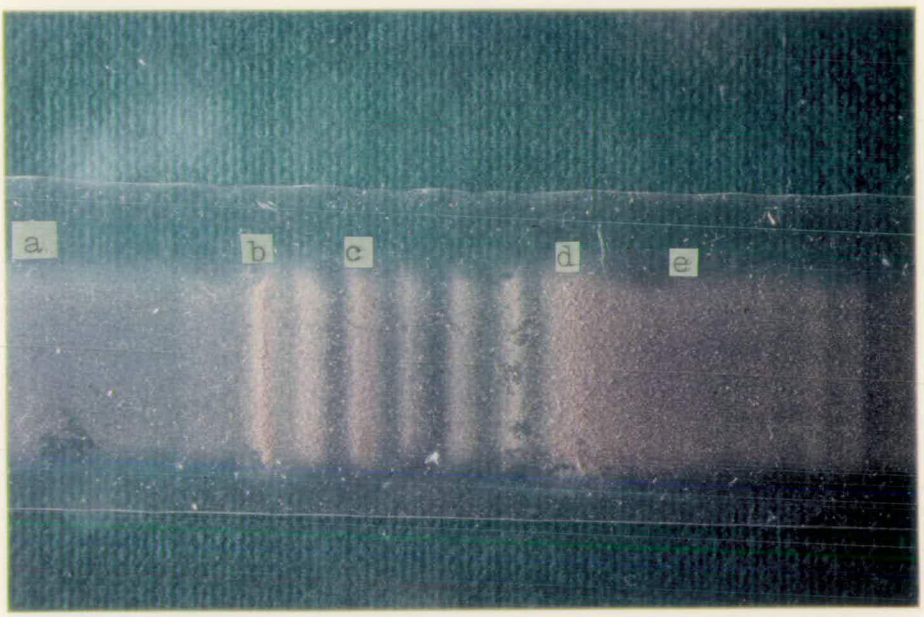


Figure 7:A spore trace obtained over one 24 hour period using the Burkard volumetric spore trap in a dairy byre.
a:00.00 hr.
b:07.30 hr.;morning unbaling of hay.
c:10.30 hr.;bedding of cattle with barley straw.
d:17.30 hr.;evening unbaling of hay and straw.
e:24.00 hr.

Absorbance patterns of spore traces

Figure 8 demonstrates a typical absorbance pattern obtained by passing a mounted trace through a Joyce Chromoscan. The two main peaks of absorbance corresponded to the times of morning foddering and evening foddering and bedding. Variations in the shapes of morning and evening peak absorbances during a period of 6 days in March 1970 are shown in Figure 9. The shape of the morning peaks was uniform but considerable variation was present in the evening when one, two or three main peaks were evident. This was probably due to the fact that groups of bales of hay and/or straw had been opened and distributed at different times. The uniformity of single peaks in the morning is probably an indication of only one unbaling operation. Also seen in Figure 9 are subsidiary peaks following the main evening peak, as for March 15th 1970. As these peaks occurred in the late evening the only apparent cause would seem to be animal movement either in close proximity to the spore-trap or throughout the byre.

b) Microscopic Examination of Spore Traces

Photographs of the regions of greatest and least density of fungal propagules (spores and hyphal fragments), particles of inorganic dust and of other debris of plant or animal origin are shown in Figure 10. Propagules of the dimensions of actinomycetes and bacteria as well as particles of inorganic dust were present in high concentrations even in the regions of least density of the larger fungal propagules. As seen in Figure 10, the numbers of fungal propagules and other particles were readily observed in the least dense regions but it was extremely difficult to enumerate propagules in the dense regions. Results from areas of peak concentration must, therefore, be regarded as approximations due to the depth of focus required to count the large numbers of propagules and other particles involved.

Some of the different types of fungal spores and hyphal fragments found on the traces can be seen in Figures 11 and 12.

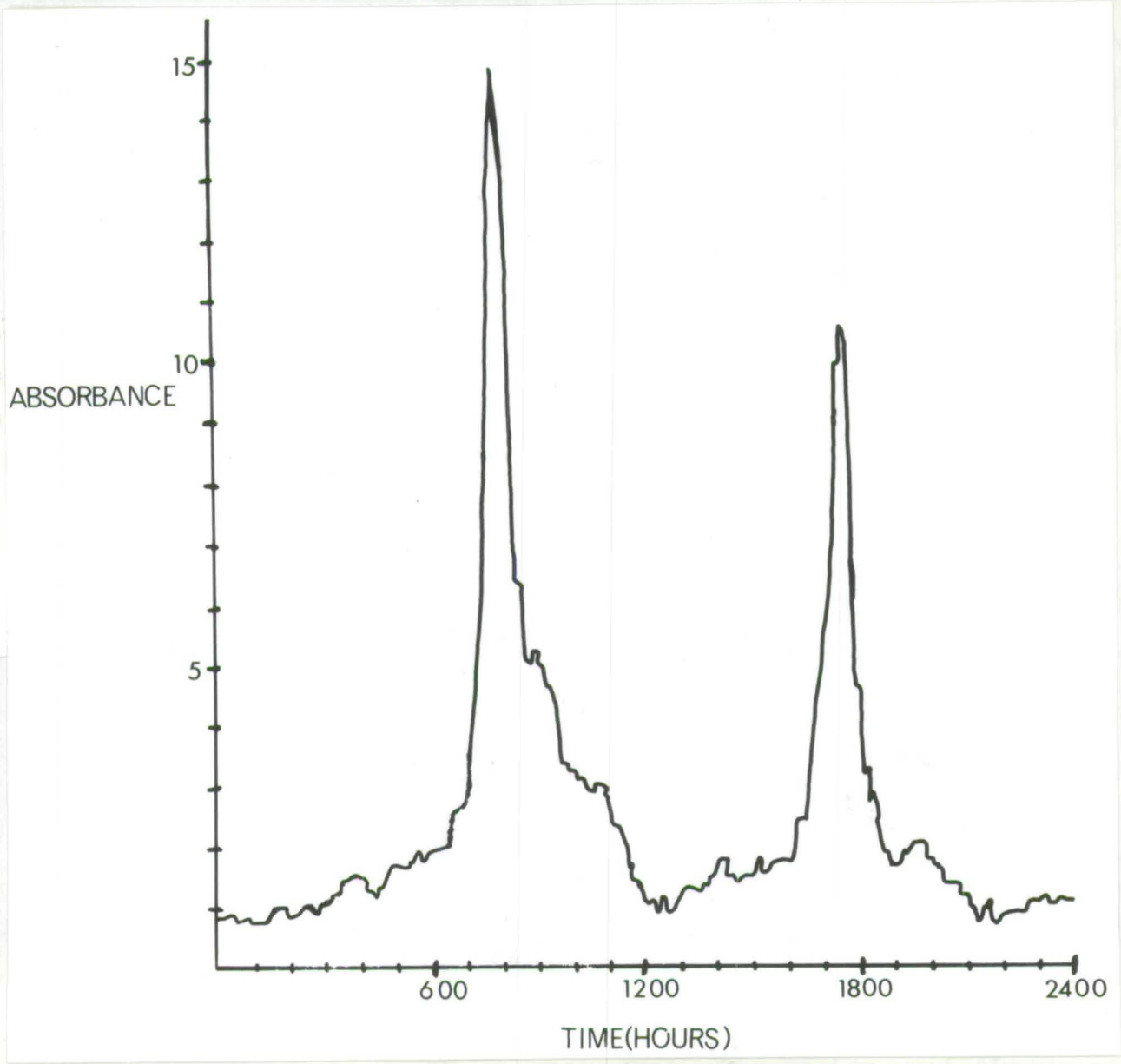
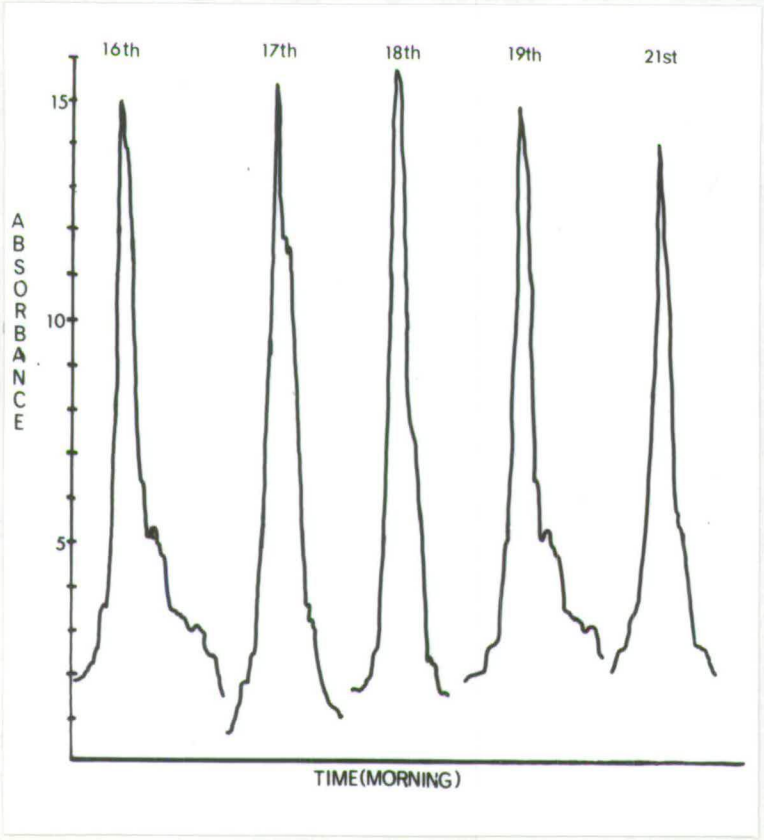


Figure 8: The absorbance pattern of a 24 hour period of trace obtained by using the Burkard volumetric spore trap, and measured on a Joyce Chromoscan.



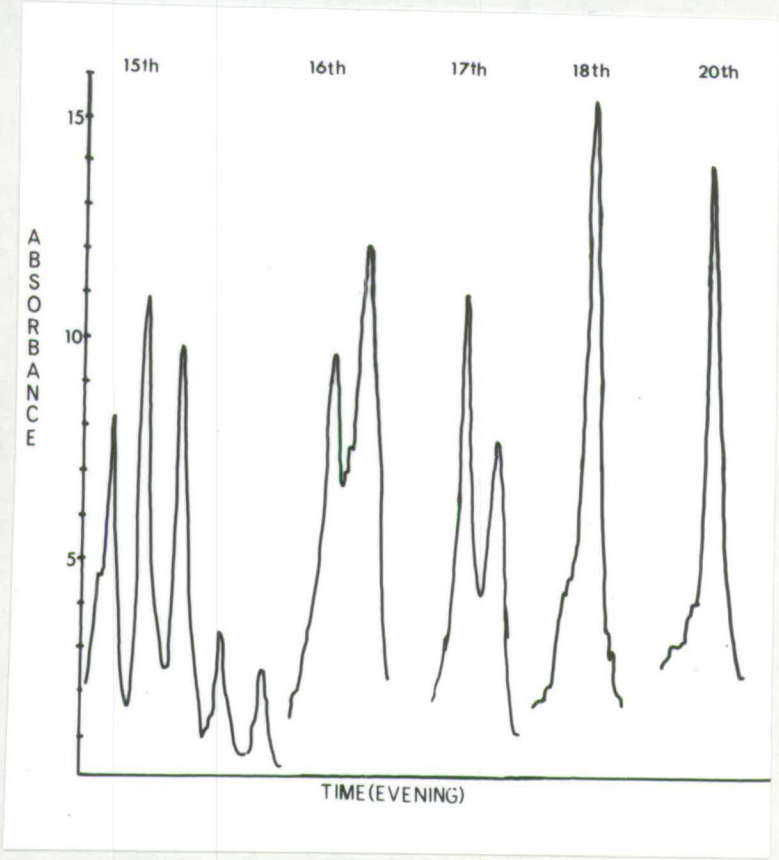


Figure 9:Variations in the absorbance patterns of spore traces, obtained by using the Burkard volumetric spore trap and measured by a Joyce Chromoscan at times of peak absorbance on five morning and five evenings in March,1970.

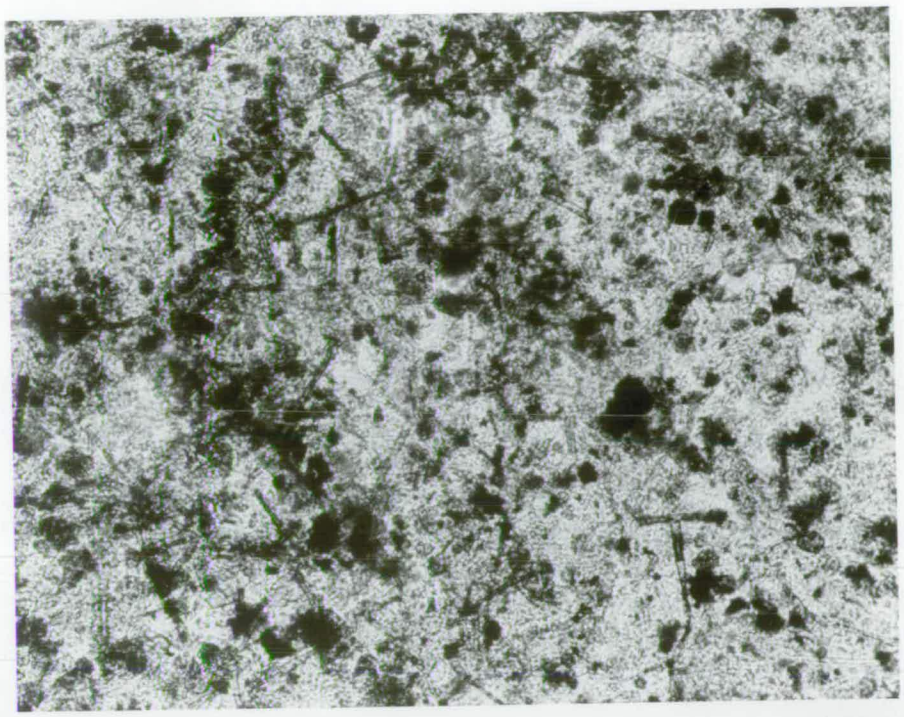


Figure 10: Microscopic appearance of spore trace obtained from Burkard volumetric spore trap demonstrating a) region of trace with dense concentration of fungal propagules, and b) region with less dense concentration.

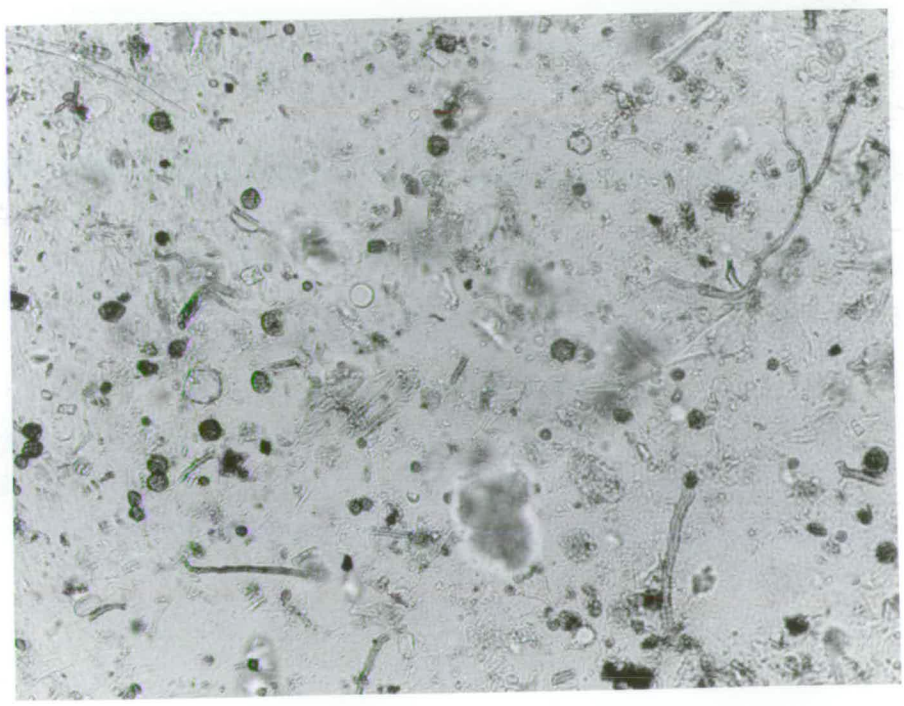




Figure 11: Fungal propagules from a portion of spore trace from the Burkard volumetric spore trap.
a. *Alternaria* spore
b. *Humicola* spore
c. Hyphal fragment
d. portion of chlorophyllous plant material.

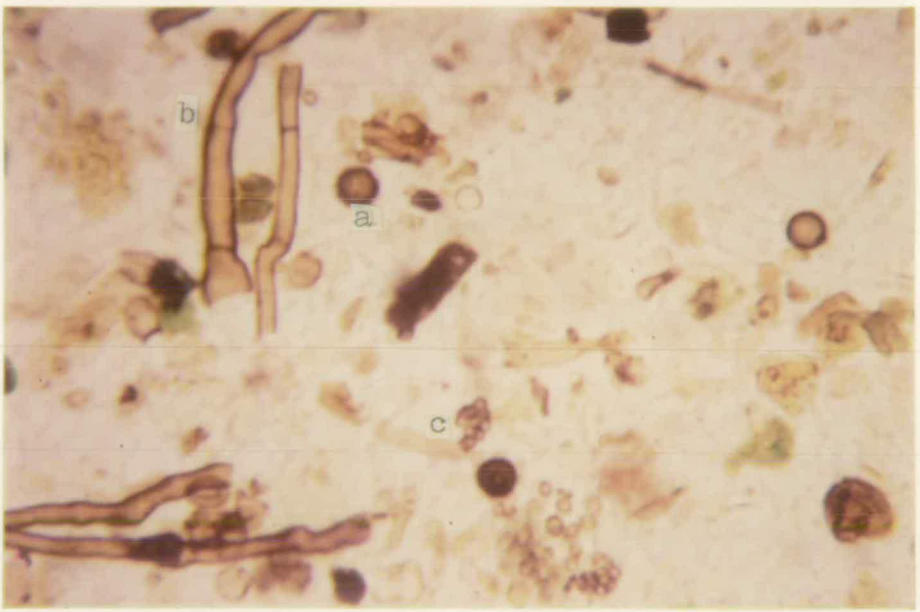


Figure 12: Fungal propagules from a portion of the spore trace from the Burkard volumetric spore trap.
a. *Humicola* spore
b. Hyphal fragment
c. *Aspergillus*/*Penicillium* type spores.

i) The numbers of fungal propagules at times of peak concentrations

The peak numbers of fungal propagules per cubic metre of air for morning and evening, over a period of 6 days, are shown in Table 1. Values for the evening of March 19th and the morning of March 20th 1970 were omitted as the particles had moved while being mounted. The peak numbers of propagules in the morning ranged from $4.07 \times 10^6/m^3$ to $7.31 \times 10^6/m^3$. The numbers of propagules/m^3, calculated from the spore trace when two or three peaks were present in the evening, were added together to give values of 3.52×10^6 to 5.92×10^6, figures equivalent to, but on average lower than those for the morning peaks.

ii) Variation in the numbers of fungal propagules per cubic metre of air, over one twenty-four hour period.

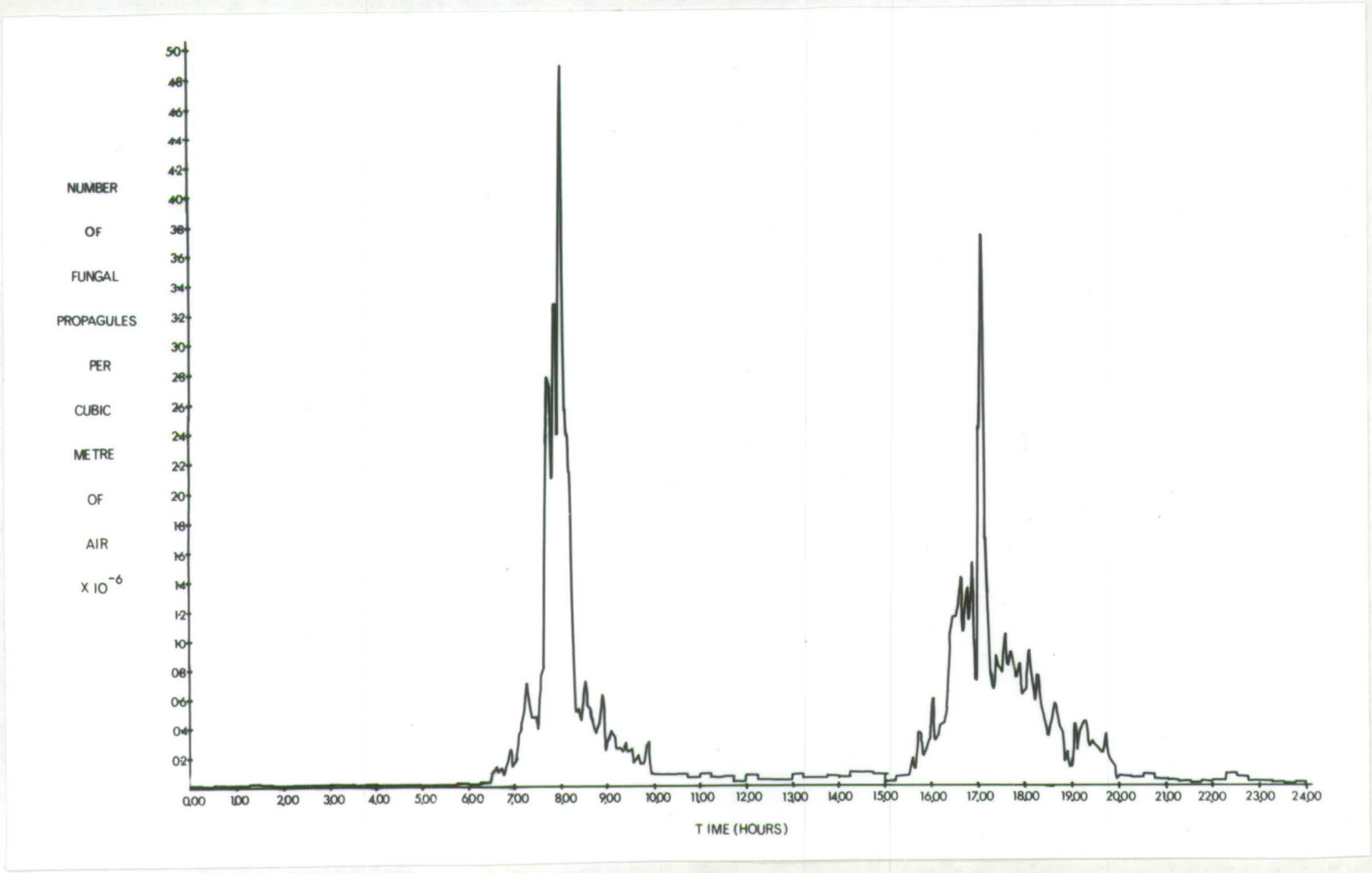
The highest recorded counts of fungal spores and hyphal fragments per cubic metre of air, for March 16th 1970, as shown in Graph 1, were associated with the times of feeding, foddering and bedding of the animals (see Figure 1). The twenty-four hour period of Graph 1 may be divided into peak and off-peak time intervals, off-peak time being that when there is an almost constant basal number of fungal propagules. Off-peak times were taken as 00.00 hr. to 06.30 hr., 10.00 hr. to 15.30 hr., and 20.00 hr. to 24.00 hr. During the peak times, 06.30 hr. to 10.00 hr. and 15.30 hr. to 20.00 hr., the numbers of fungal propagules are expressed as the number per cubic metre of air, calculated from microscope counts per $^1/16$ hr. For off-peak times, four of the results per $^1/16$ hr. were averaged to give a value per $^1/4$ hr., to allow these values to be easily seen on the same scale as the very high values at peak concentration. The average numbers of fungal propagules per cubic metre of air over the five main time intervals are shown in Table 2, together with the range of values obtained within each time interval.

Peak Time Intervals

The greatest numbers (peak values) of fungal propagules were recorded

	Number of fungal propagules per cubic metre of air X 10^{-6}		
Date	Morning Peaks	Evening Peaks	Total
15/3/70		1.07; 2.65; 2.20	5.92
16/3/70	4.75	1.25; 3.92	5.17
17/3/70	4.07	2.53; 2.41	4.94
18/3/70	4.53	4.60	4.60
19/3/70	7.31	NO RESULT	
20/3/70	NO RESULT	3.52	3.52
21/3/70	7.06		
AVERAGE	5.55		4.83

Table 1: The numbers of fungal propagules per cubic metre of air, isolated from a dairy byre at times of peak propagule concentration, obtained over a six-day period by a Burkard volumetric spore trap.



Graph 1: The numbers of fungal propagules isolated per cubic metre of air using a Burkard volumetric spore trap over one 24 hour period in a dairy byre.

	Time Interval	Number of fungal propagules per metre^3 X 10^{-3} Average	Range
MORNING PEAK	06.30hr.-10.00hr.	768	77 - 4,897
EVENING PEAK	15.30hr.-20.00hr.	658	53 - 3,746
TOTAL PEAK		706	
OFF-PEAK TIMES	00.00hr.-06.30hr.	19	3 - 62
	10.00hr.-15.30hr.	70	18 - 154
	20.00hr.-24.00hr.	45	6 - 133
TOTAL OFF-PEAK		43	
TOTAL PEAK+OFF-PEAK		264	

Table 2: The average number of fungal propagules per cubic metre of air during time intervals of peak and off-peak concentrations, calculated from a spore trace obtained by using the Burkard seven-day spore trap, over one twenty-four hour period.

at approximately 08.00 hr. to 08.03 hr. and 17.04 hr. to 17.07 hr. and were 4,897 x $10^3/m^3$ and 3,746 x $10^3/m^3$ respectively. The morning peak value and the average number of propagules/m^3 during the morning peak time interval were higher than the corresponding evening values (Table 2). The over-all peak average was 706 x 10^3 fungal propagules/m^3.

Off Peak Time Intervals

The average number of spores per cubic metre of air between 10.00 hr. and 15.30 hr. (70 x 10^3) was higher than the average value for the two other off-peak times (Table 2). The equivalent evening time interval, 20.00 hr. to 24.00 hr., gave an average of 45 x 10^3. The very low average value of 19 x $10^3/m^3$ between 00.00 hr. and 06.30 hr. correlates with the length of time from the last previous release of large numbers of propagules into the atmosphere (i.e. the previous day at approximately 17.30 hr.). There is again an indication of the release of larger numbers of propagules in the morning than in the evening but this is complicated by the fact that work, with a consequent disturbance of air and circulation of propagules, was being done in the byre after the morning milking. This was reflected in the banding patterns on the spore trace, during the day. No work is being done between approximately 17.30 hr. the one day and 05.15 hr. the next. The range of values within each off-peak time interval (Table 2) correlates with the average value for each interval, further substantiating the indications of these results. The over-all off-peak average is 43 x 10^3 fungal propagules /m^3 and the average for the complete twenty-four hour period is 264 x 10^3 fungal propagules/m^3 (Table 2)

Decrease in the numbers of fungal propagules with time from peak value concentration.

The number of fungal propagules at the peak evening value on Graph 1 was taken as 100% and values obtained during a 1/16 hr. period at 30-minute intervals for up to 6 hours after the peak value, were expressed as a percentage of the peak value. These results are shown on Graph 2.

After 30 minutes the numbers have dropped to 25% of the original vlaue. after 1 hour to 24% and after 6 hours to 0.8%.

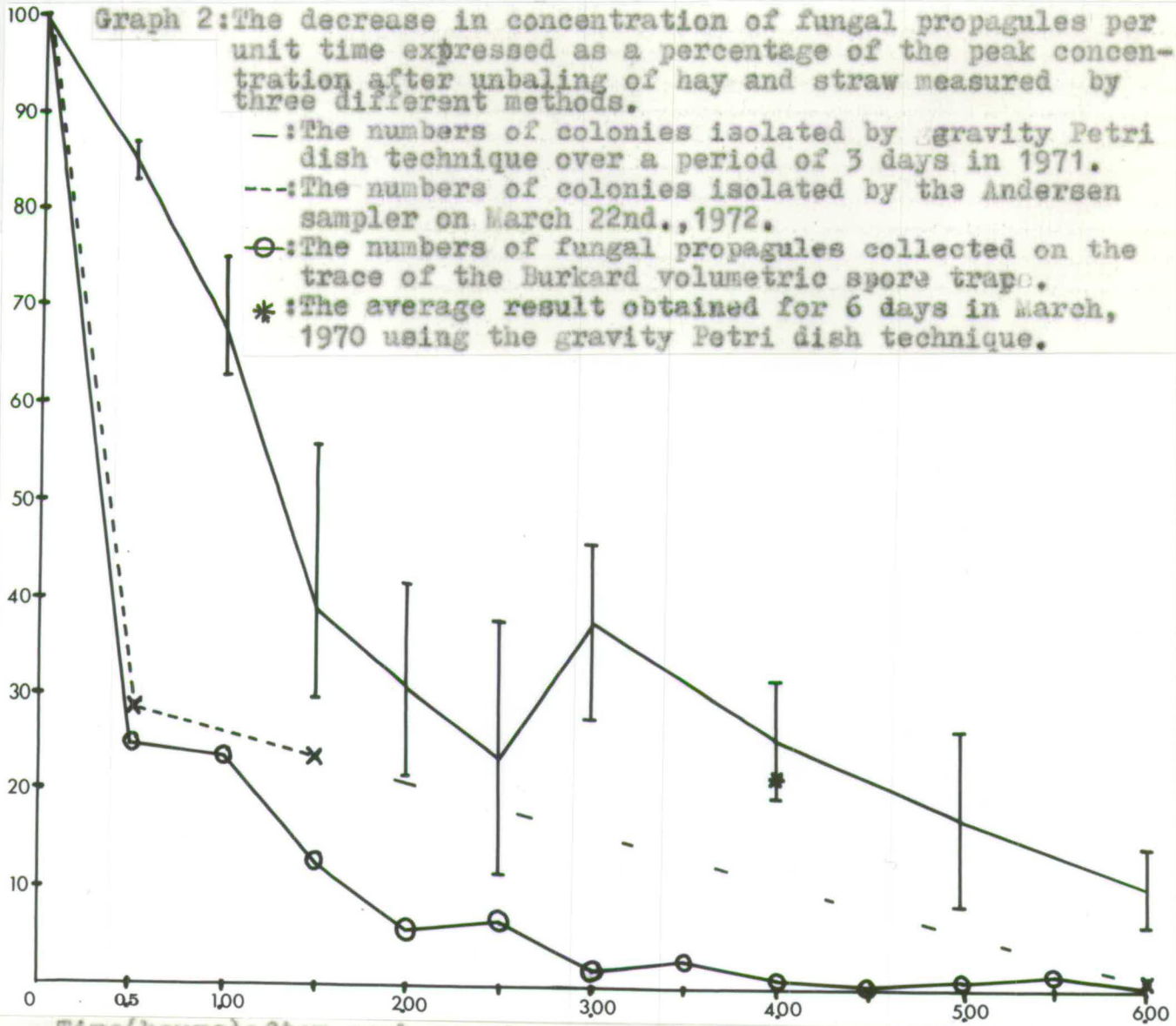
Graph 2: The decrease in concentration of fungal propagules per unit time expressed as a percentage of the peak concentration after unbaling of hay and straw measured by three different methods.

—: The numbers of colonies isolated by gravity Petri dish technique over a period of 3 days in 1971.

---: The numbers of colonies isolated by the Andersen sampler on March 22nd., 1972.

-O-: The numbers of fungal propagules collected on the trace of the Burkard volumetric spore trap.

*: The average result obtained for 6 days in March, 1970 using the gravity Petri dish technique.

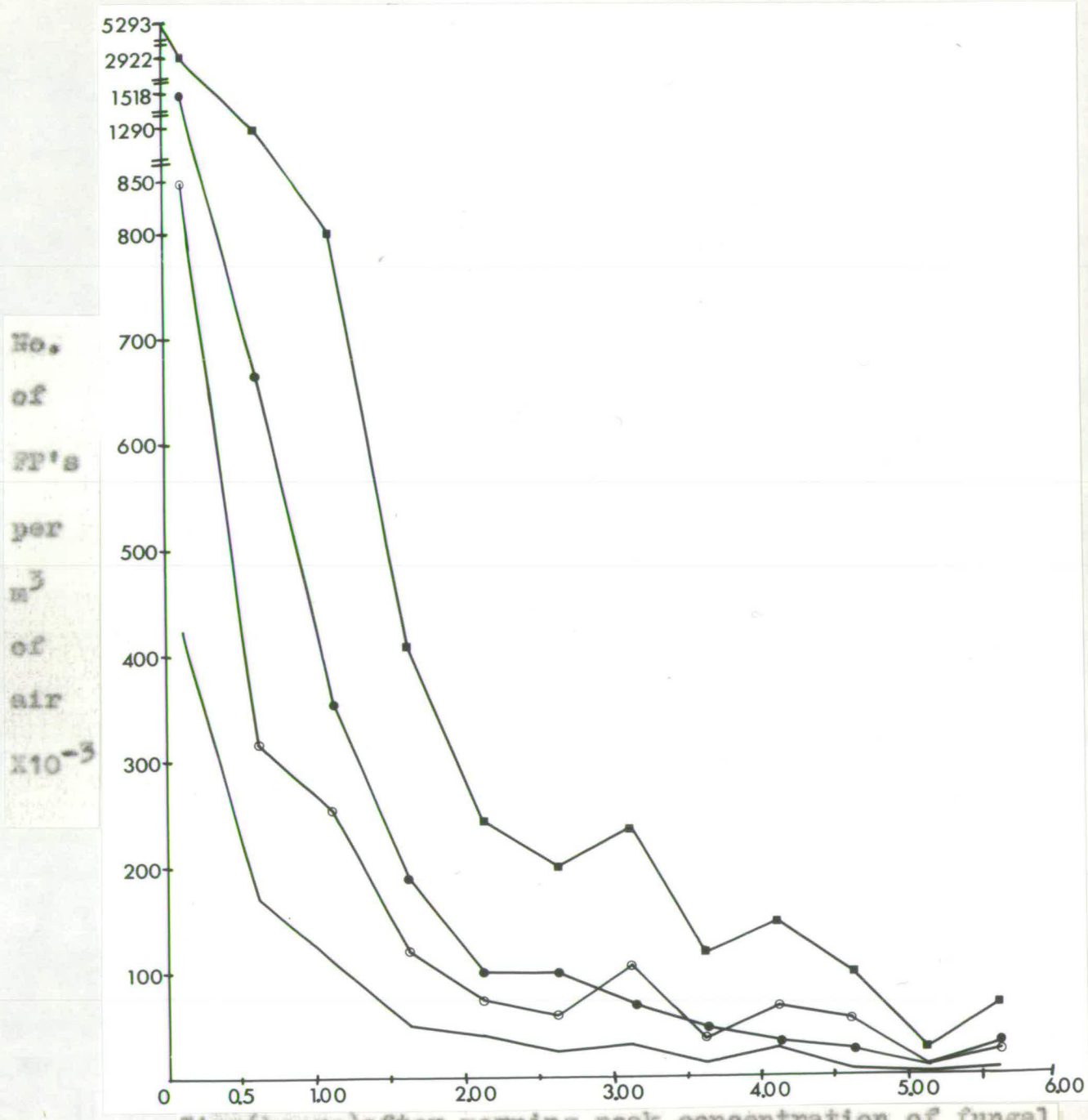


iii) The distribution of different types of fungal propagule after three morning and three evening peak concentrations

The numbers of fungal propagules averaged over three days

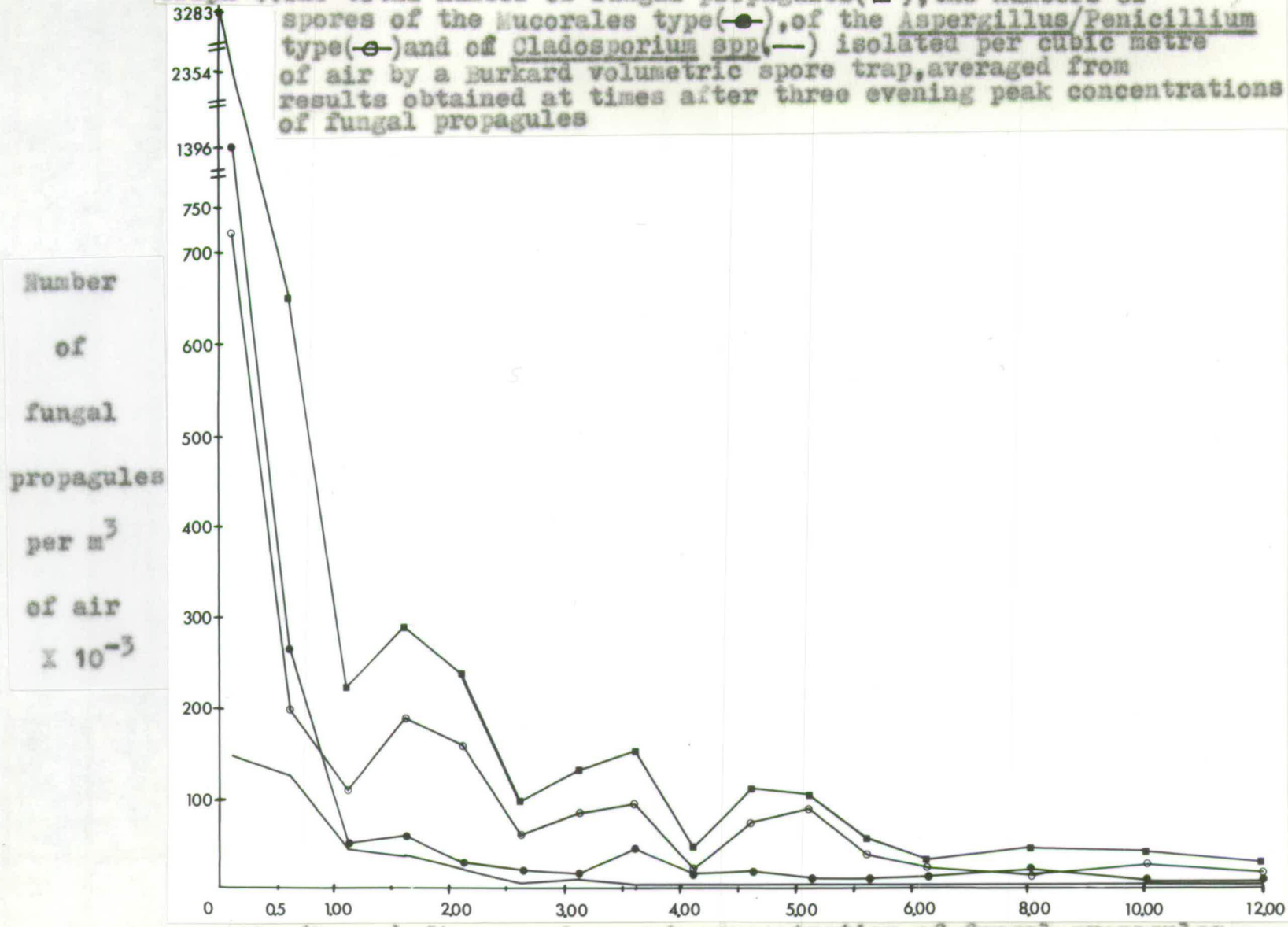
The average numbers of fungal propagules/m^3 calculated from counts obtained from 3 morning and 3 evening traces from the Burkard volumetric spore trap are shown in Graphs 3 and 4. Morning results were obtained for the 16th, 17th and 21st March, 1970, and evening values were for the 16th, 17th and 20th March, 1970. The total number of fungal propagules, the numbers of spores of Aspergillus/Penicillium type, of Cladosporium spp. and of the Mucorales type were recorded at alternate 15 minute intervals for up to 5h.45m. after morning peak concentration and for up to 12h. after evening peak concentration. The numbers of the remaining types of fungal propagules are also shown in Graphs 3 and 4 and include the following: spores of Humicola spp. (H. lanuginosa, H. insolens and H. stellata), sporangial heads of Mucoraceous fungi (Absidia spp., Mucor spp. and Rhizopus spp.), spores of the genera Trichoderma, Fusarium, Erysiphe, Alternaria, Trichothecium, Acremoniella and Epicoccum, cells of yeasts and hyphal fragments. Numerous fragments of plant material were also seen as well as small refractile bodies which were of the dimensions of spores of actinomycetes or of bacterial cells. Neither of these was included in the counts shown in Graphs 3 and 4.

The average peak morning concentration was 5,293 x 10^3 fungal propagules/m^3 (Graph 3) and the corresponding evening value was 3,283 x $10^3/m^3$ (Graph 4). Spores of the Mucorales type made up the largest proportion of fungal propagules for up to 2h. 30m. to 2h. 45m. (2h. 30m.) after the morning peak but for up to only 30m. after the evening peak. At 0-15m. after peak concentrations for morning and evening the numbers of spores of the Mucorales were 1,518 x $10^3/m^3$ and 1,396 x $10^3/m^3$ respectively. The numbers of Aspergillus/Penicillium-type spores, the next most common at that time interval had values of 848 x $10^3/m^3$ and 724 x $10^3/m^3$. For 1h. to 12h. after peak concentrations there were more Aspergillus/Penicillium-



Graph 3: The total number of fungal propagules(FP's)(—■—), the numbers of spores of the Mucorales type(—●—), of the Aspergillus/Penicillium type(—⊖—) and of Cladosporium spp.(——) isolated per cubic metre of air by a Burkard volumetric spore trap, averaged from results obtained at times after three morning peak concentrations of fungal propagules.

Graph 4: The total number of fungal propagules(-■-), the numbers of spores of the Mucorales type(-●-), of the Aspergillus/Penicillium type(-⊖-) and of Cladosporium spp(—) isolated per cubic metre of air by a Burkard volumetric spore trap, averaged from results obtained at times after three evening peak concentrations of fungal propagules



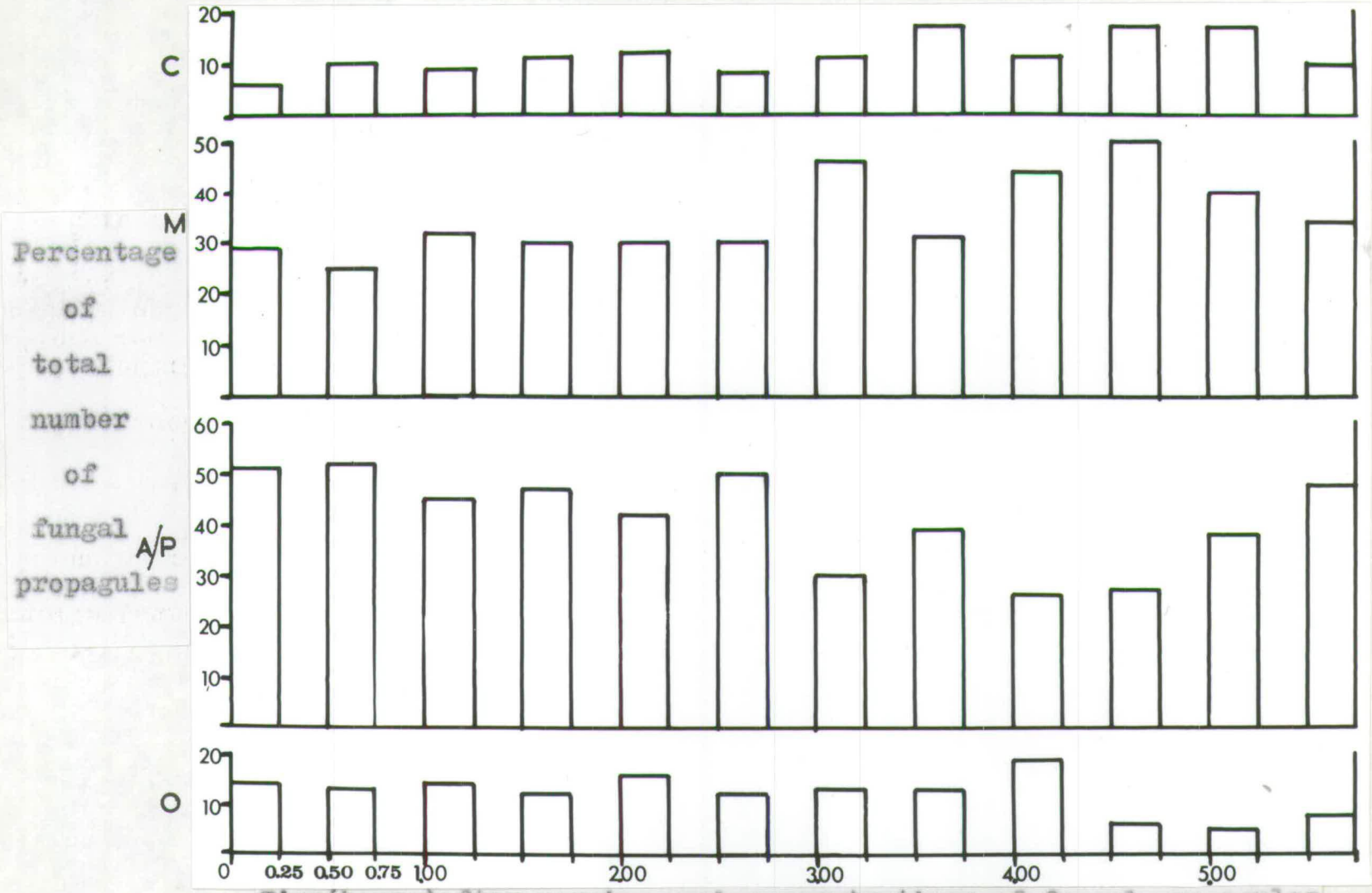
type spores than spores of the Mucorales type with the exception of the count at 8h. after morning peak concentration when Aspergillus/Penicillium-type spores were marginally more common, by 8 x $10^3/m^3$. Spores of Cladosporium spp. were considerably less common than those of either Mucorales or Aspergillus/Penicillium-type and had concentrations of 423 x $10^3/m^3$ and 145 x $10^3/m^3$, 0-15m. after morning and evening peaks of concentration, respectively.

The relative proportion of different spore types.

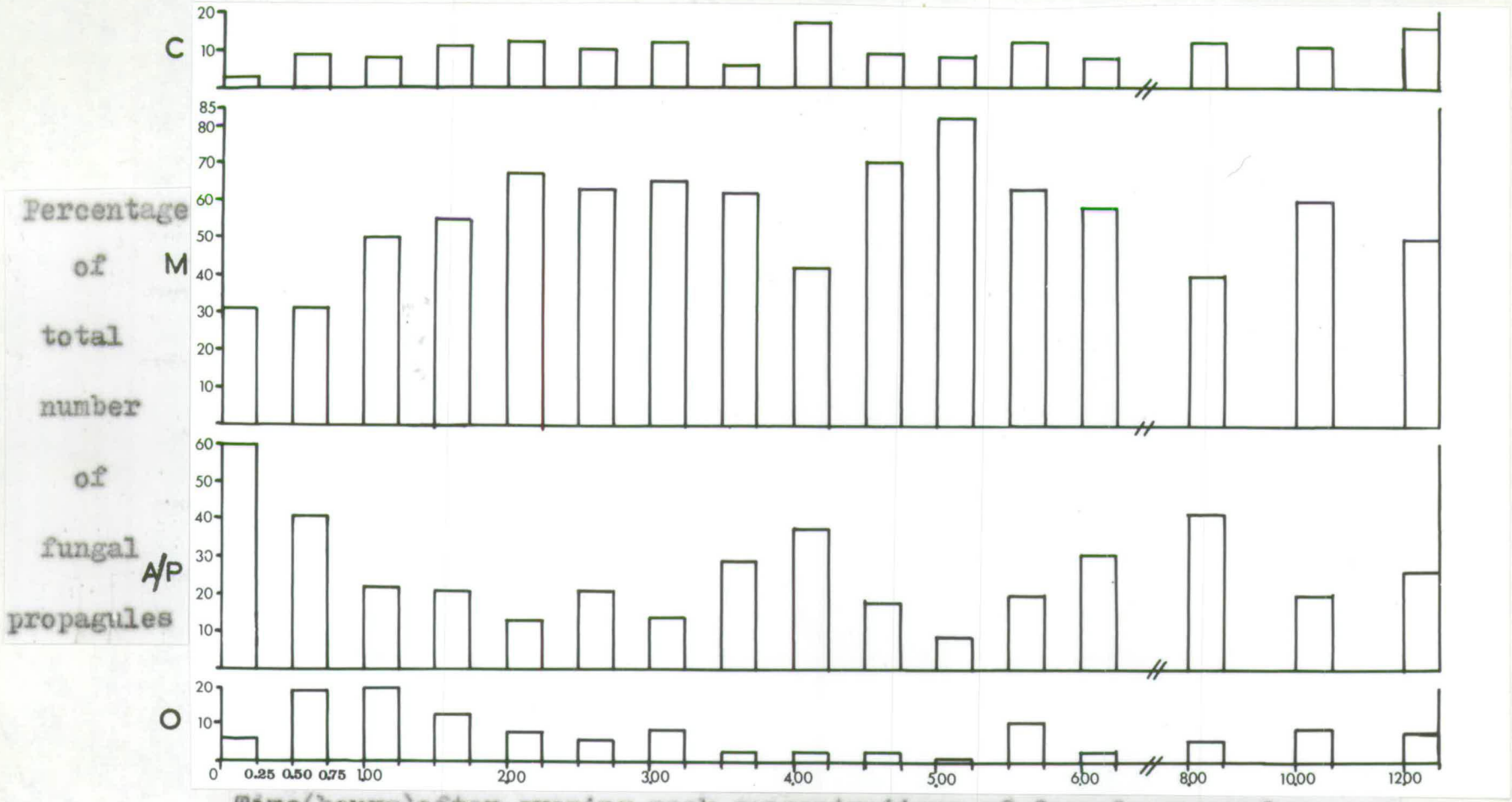
The proportions of spores of Cladosporium spp., Mucorales type and Aspergillus /Penicillium-type present at a particular time interval, expressed as a percentage of the total at that time interval are shown in Graphs 5 and 6. The relative proportions of other fungal propagules are also shown. There was more variation in the proportions of different types of propagule at given times after peak evening concentrations (Graph 6) than after morning concentrations (Graph 5). In the evenings, the proportion of spores of the Mucorales type decreased sharply from 60% at 0 to 15m. to 13% at 2h. after peak concentration. A corresponding increase in the proportion of Aspergillus/Penicillium-type spores during the same time interval was from 31% to 67%. For the corresponding time interval after the morning peaks, the proportion of Aspergillus/Penicillium-type spores rose only from 29% to 30% and that of the Mucorales type, after an initial increase from 51% to 52% within the first 30 minutes, dropped only to 42%.

Graph 7 demonstrates the relationship between the proportion of Aspergillus/Penicillium-type spores and that of Mucorales type spores over both the morning and evening post-peak periods. This shows that the concentration of Aspergillus/Penicillium-type spores is inversely proportional to that of spores of the Mucorales type. It also illustrates a higher proportion of Mucoraceous spores with a correspondingly lower proportion of Aspergillus/Penicillium-type spores in the morning than in the evening.

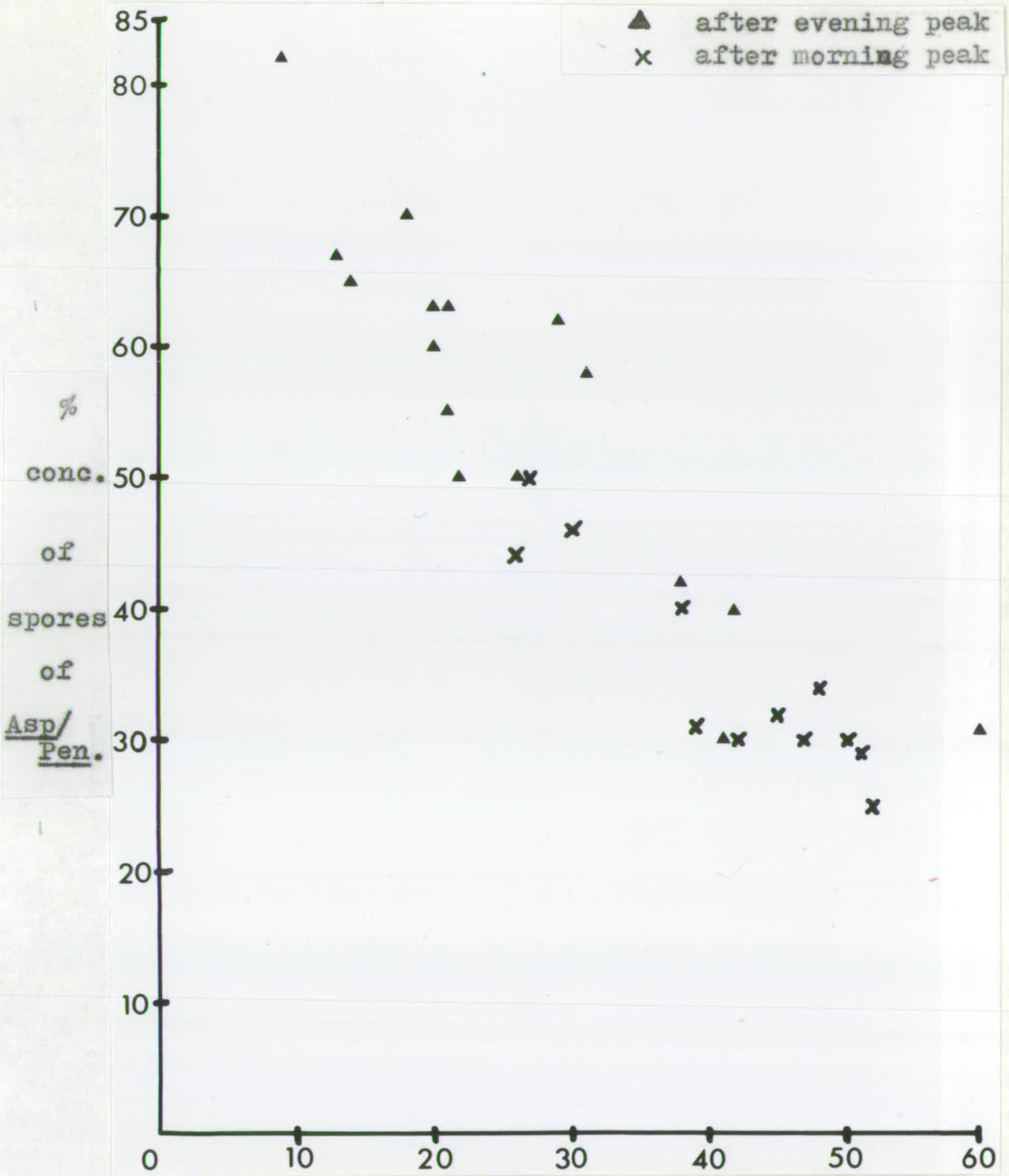
Spores other than the three main types discussed above showed variations in their relative proportions with time (Graphs 5 and 6) but did not rise



Graph 5:The relative concentrations of types of fungal propagules expressed as a percentage of the total number of fungal propagules per cubic metre of air in a dairy byre at alternate 15 minute intervals after peak morning concentration on three days. Types of spore-C=Cladosporium, M=Mucorales, A/P=Aspergillus/Penicillium, O=Others.



Graph 6: The relative concentrations of types of fungal propagules expressed as a percentage of the total number of fungal propagules per cubic metre of air in a dairy byre at alternate 15 minute intervals after peak evening concentration on three days. Types of spore-C=Cladosporium, M=Mucorales, A/P=Aspergillus/Penicillium, O=Others.



Graph 7:The percentage of spores of the Aspergillus/Penicillium type(Asp/Pen) v.the percentage of spores of members of the Mucorales at equivalent times after morning and evening peak concentrations of fungal propagules.

above 17% in the morning or evening and their minimal values were 3% in the evening and 6% in the morning (0-15m. after peak concentrations in both cases).

Variations in the numbers of different spore types at equivalent times after peak concentration on three days.

Cladosporium spp.

When Graphs 8 and 9 are compared, it can be seen that the initial concentration of spores of Cladosporium spp. was higher for all three mornings than for the evenings but that after 5 hours the values were equivalent. The pattern of decrease in concentration is more consistent and the decrease more rapid for the mornings than for the evenings. The average of the evening values (Graph 4) shows a much simpler picture for the first 2 hours than that exhibited when the individual results for three days are examined.

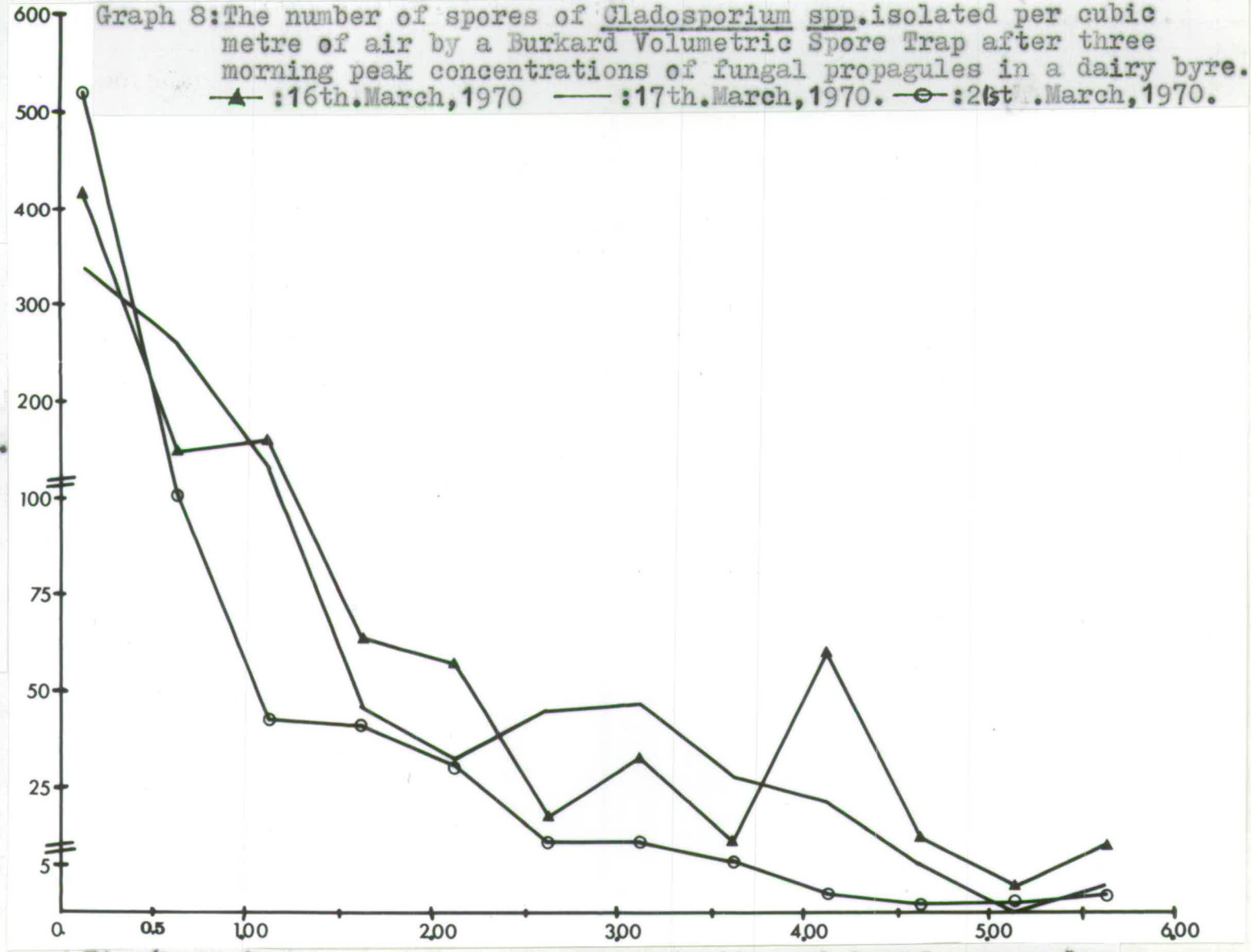
Mucorales type

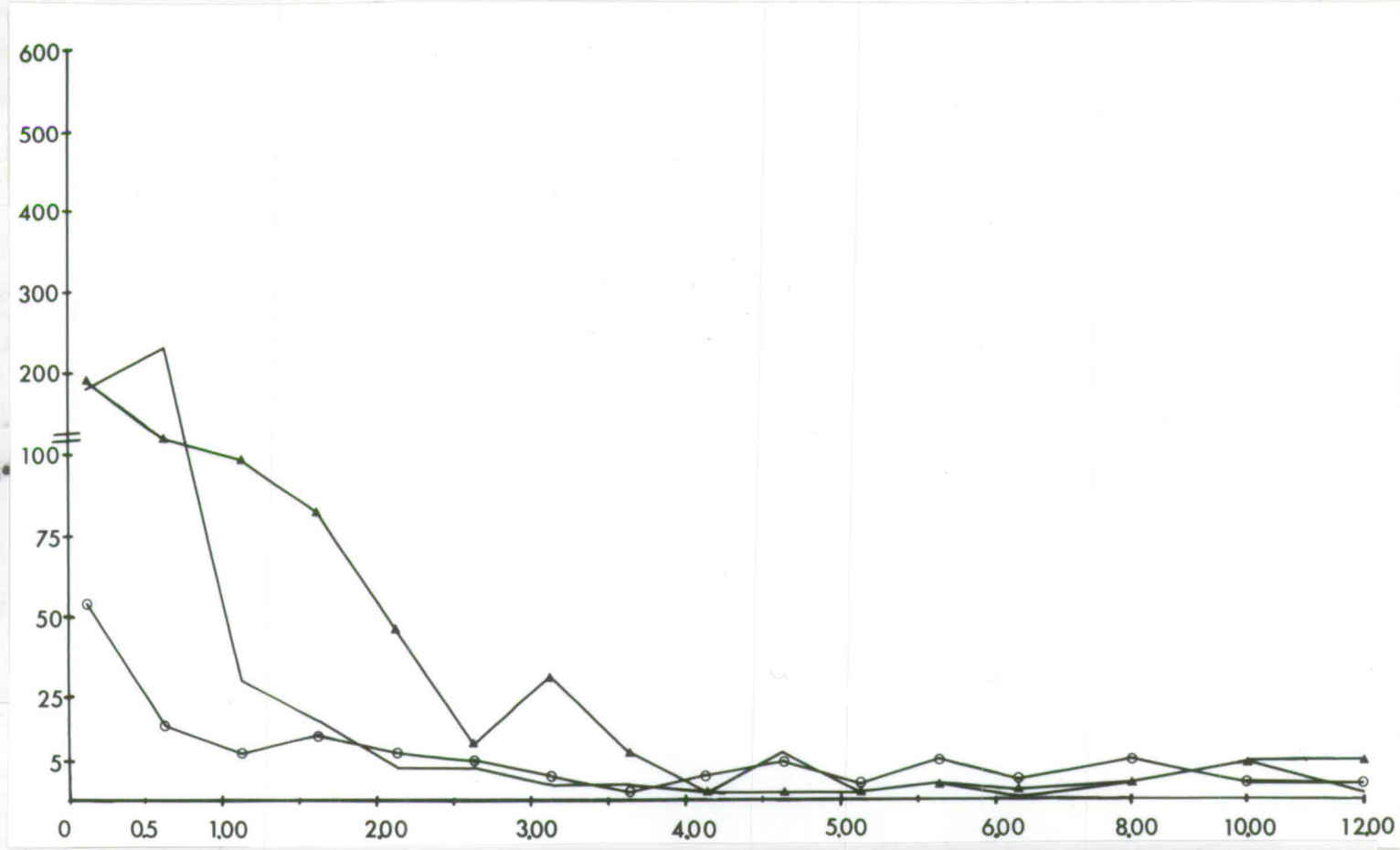
The decrease in the numbers of spores of the Mucorales type in the morning (Graph 10) is consistent over the three days but in contrast to that for spores of Cladosporium spp., is less sharp in the morning than in the evening between 0-15m. and 1h. after peak concentration (Graphs 10 and 11). There were higher values over the first 2 hours, in the morning, although after 5 hours the values were equivalent to those of the evening, indicating a greater decrease during the 5 hours of the morning than during the equivalent time in the evening. Increases in the evening values for the 16th and 17th at 1h.30m., and for the 16th at 3h.30m after peak concentrations are exaggerated by the scale used but demonstrate the day-to-day variation in values.

Aspergillus/Penicillium spp.

The pattern of decrease in the numbers of Aspergillus/Penicillium-type spores in the morning (Graph 12) is in contrast to that of both Cladosporium spp. and the Mucorales type in showing considerable variation. One common factor is a sharp peak 3 hours after peak concentration, which is not accompanied by an increase in the numbers of spores of Cladosporium spp. or those

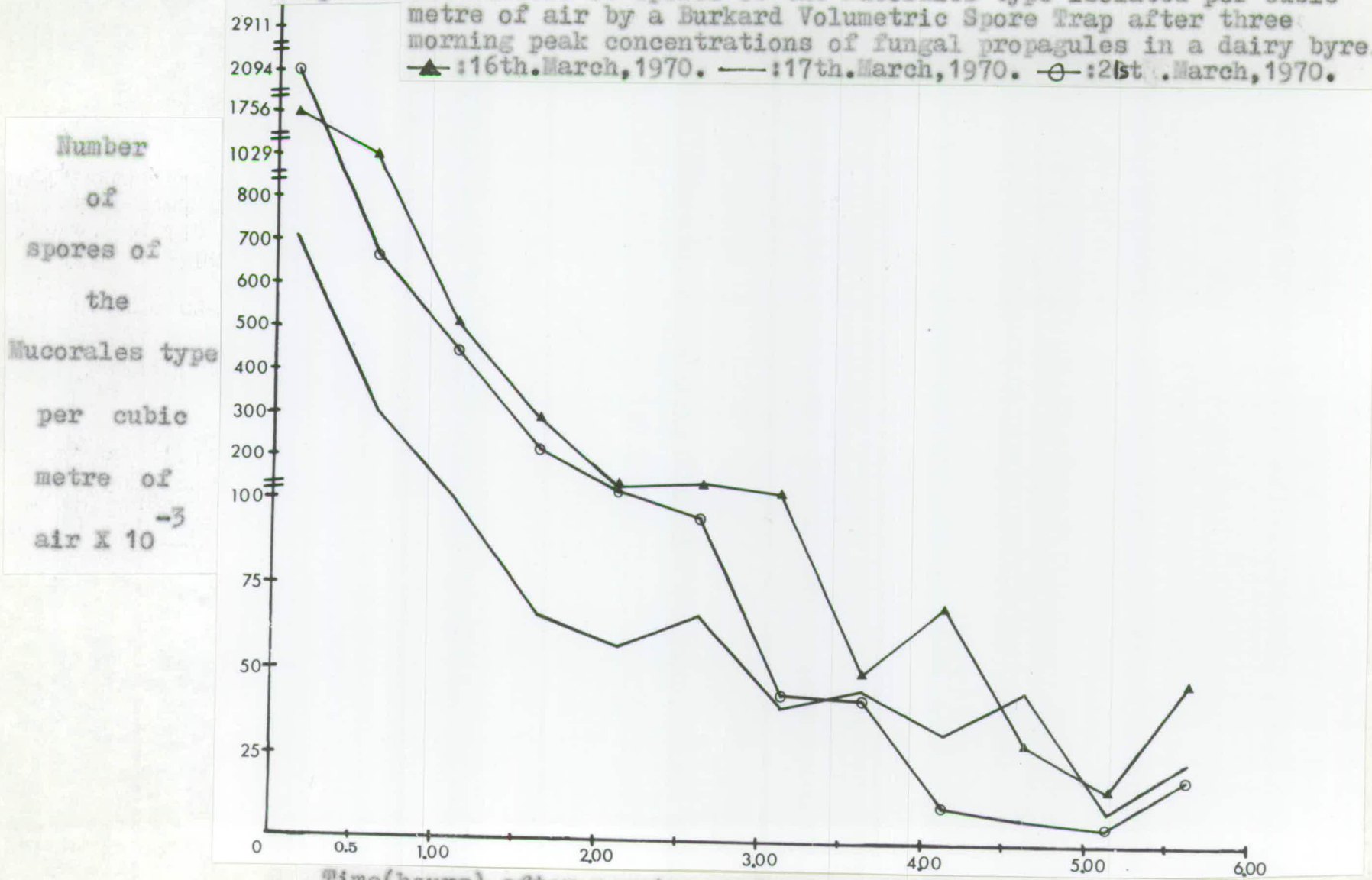
Graph 8: The number of spores of Cladosporium spp. isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three morning peak concentrations of fungal propagules in a dairy byre.
—▲— :16th.March,1970 —— :17th.March,1970. —o— :21st.March,1970.

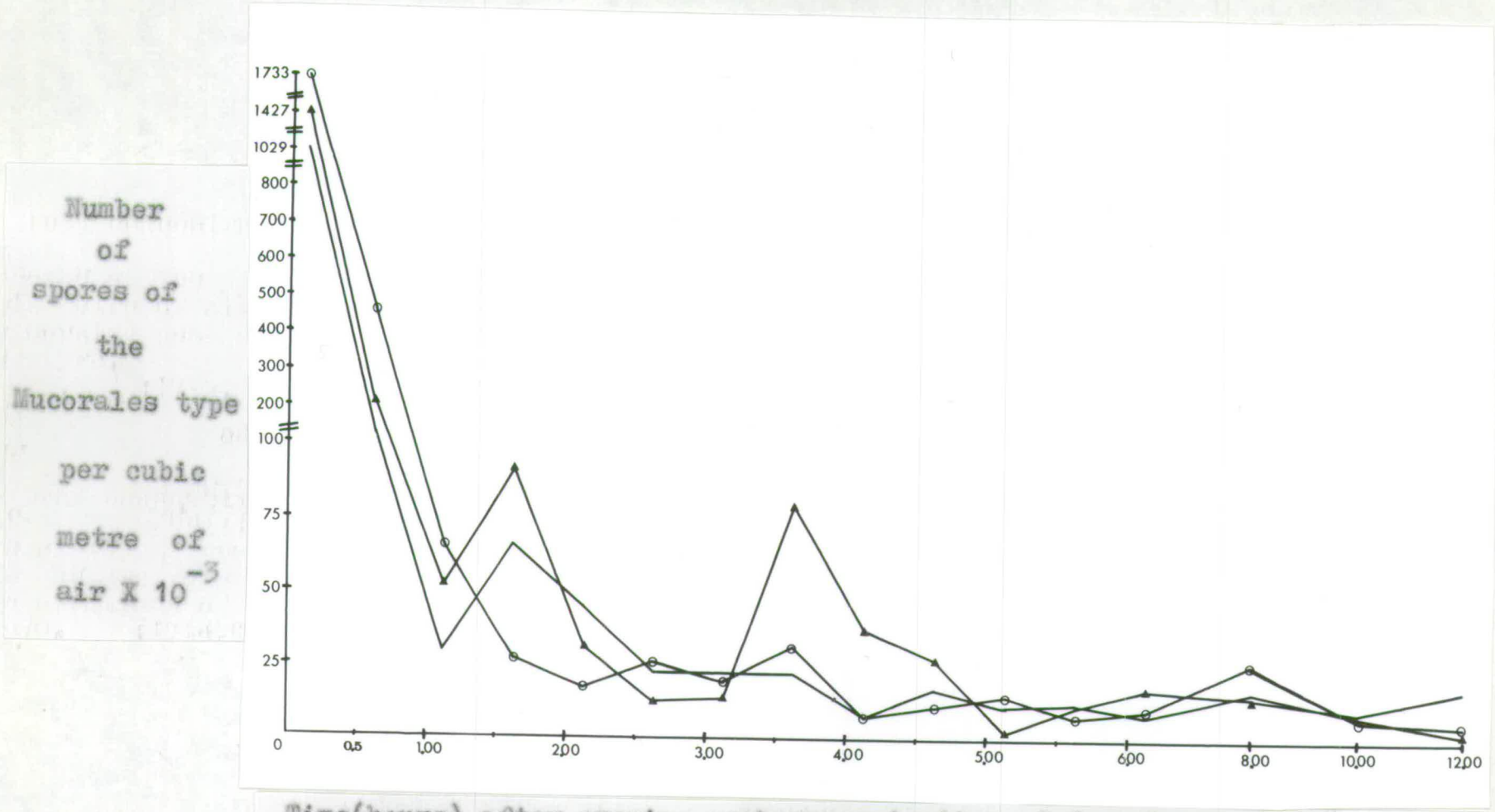




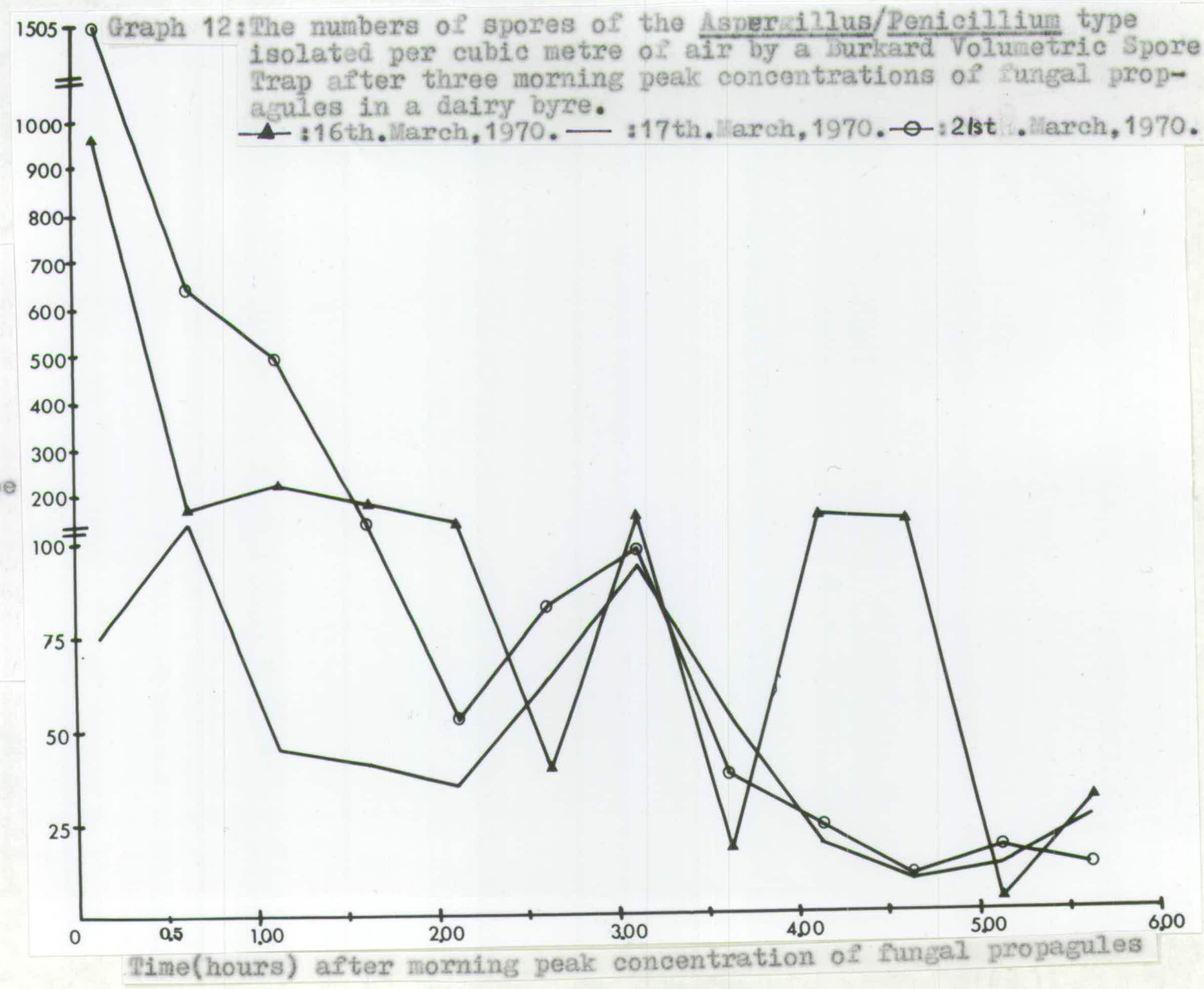
Graph 9:The number of spores of Cladosporium spp.isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three evening peak concentrations of fungal propagules in a dairy byre.
—▲— :16th.March,1970. —— :17th.March,1970. —⊖— :20th.March,1970.

Graph 10: The numbers of spores of the Mucorales type isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three morning peak concentrations of fungal propagules in a dairy byre.
▲—▲ :16th.March,1970. —— :17th.March,1970. —O— :21st.March,1970.





Graph 11: The number of spores of the Mucorales type isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three evening peak concentrations of fungal propagules in a dairybbyre.
▲ :16th.March,1970. — :17th.March,1970. —o— :20th.March,1970.


Graph 12: The numbers of spores of the Aspergillus/Penicillium type isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three morning peak concentrations of fungal propagules in a dairy byre.
▲ :16th.March,1970. — :17th.March,1970. -o- :21st.March,1970.
Number of spores of Aspergillus / Penicillium type per cubic metre of air X 10^-3
1505
1000
900
800
700
600
500
400
300
200
100
75
50
25
0
0.5
1.00
2.00
3.00
4.00
5.00
6.00
Time(hours) after morning peak concentration of fungal propagules

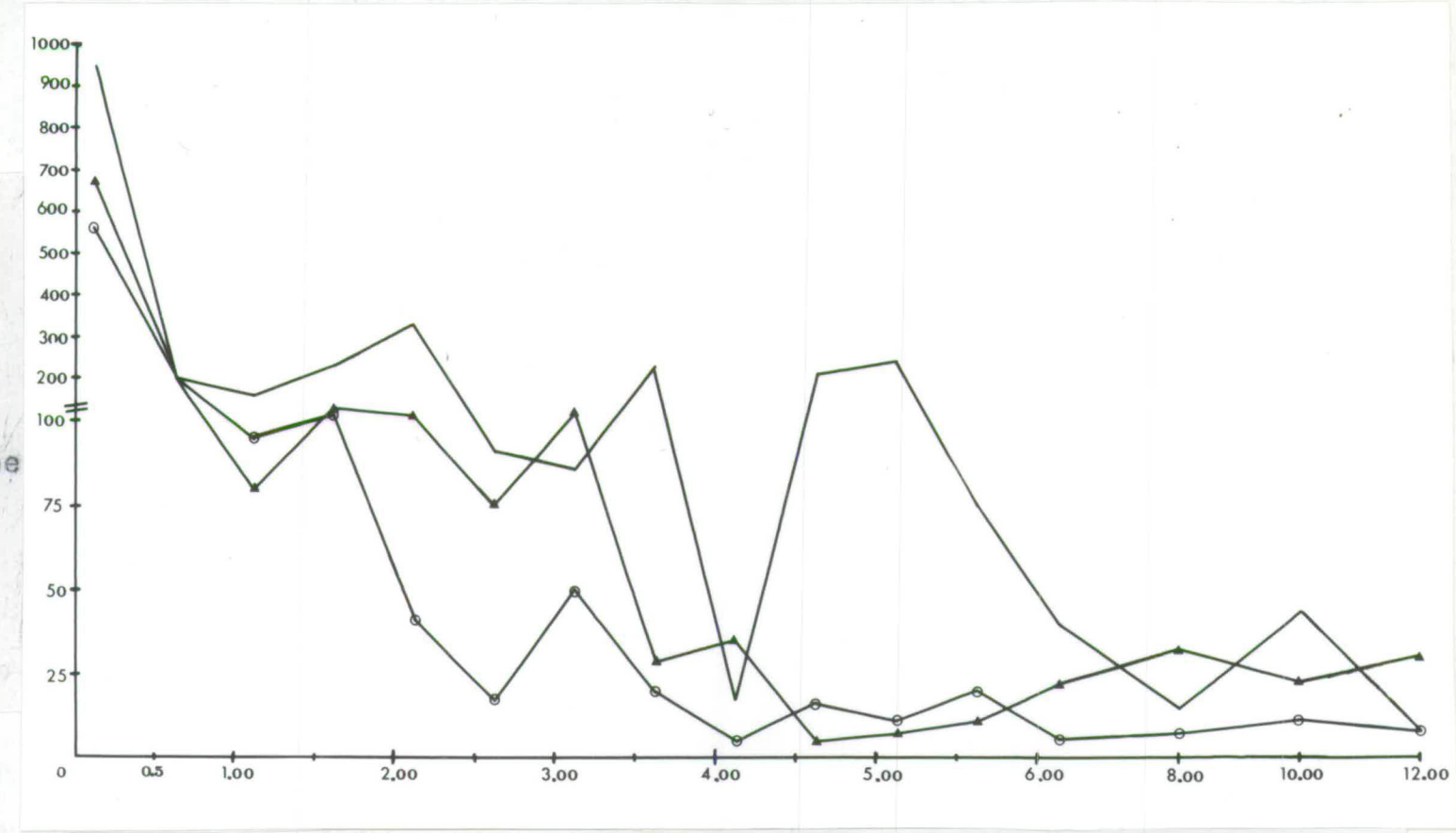
of the Mucorales. After 5 hours the three morning values for Aspergillus/Penicillium-type spores are of the same order but there is a considerable variation in the evening (Graph 13) from 2 hours onwards, which is still present after 5 hours. The numbers of Aspergillus/Penicillium-type spores for the morning of the 16th give high values at 4h. and 4h30m. and for the evening of the 17th a similar increase was evident 4h.30m. and 5h. after peak concentration. These values account for the increase in average numbers of Aspergillus/Penicillium-type spores at these times, shown in Graphs 3 and 4 and are largely responsible for the increases in total counts for the individual days shown in Graphs 14 and 15. Even 6 hours to 12 hours after the evening peak concentration, there was still considerably more variation in the numbers of Aspergillus/Penicillium-type spores than in the numbers of those of Cladosporium spp. or Mucorales type at equivalent times.

Total numbers of fungal propagules

Over the three days studied, the decreases in total numbers of fungal propagules in the mornings showed a similar pattern (Graph 14) as did the decreases in the three evening values. The morning decreases were more rapid over the first 15 minutes than those of the evening (Graph 15) but between 15 minutes and 1 hour after peak concentration the rate of decrease in the morning was less than in the evening, reflecting the results obtained for the numbers of spores of the Mucorales and Aspergillus/Penicillium-type. Peaks already described, due to increases in the numbers of spores of Mucorales or Aspergillus/Penicillium-type spores, were evident in values for both mornings and evenings.

The proportion of different types of fungal propagule after morning and evening peak concentrations.

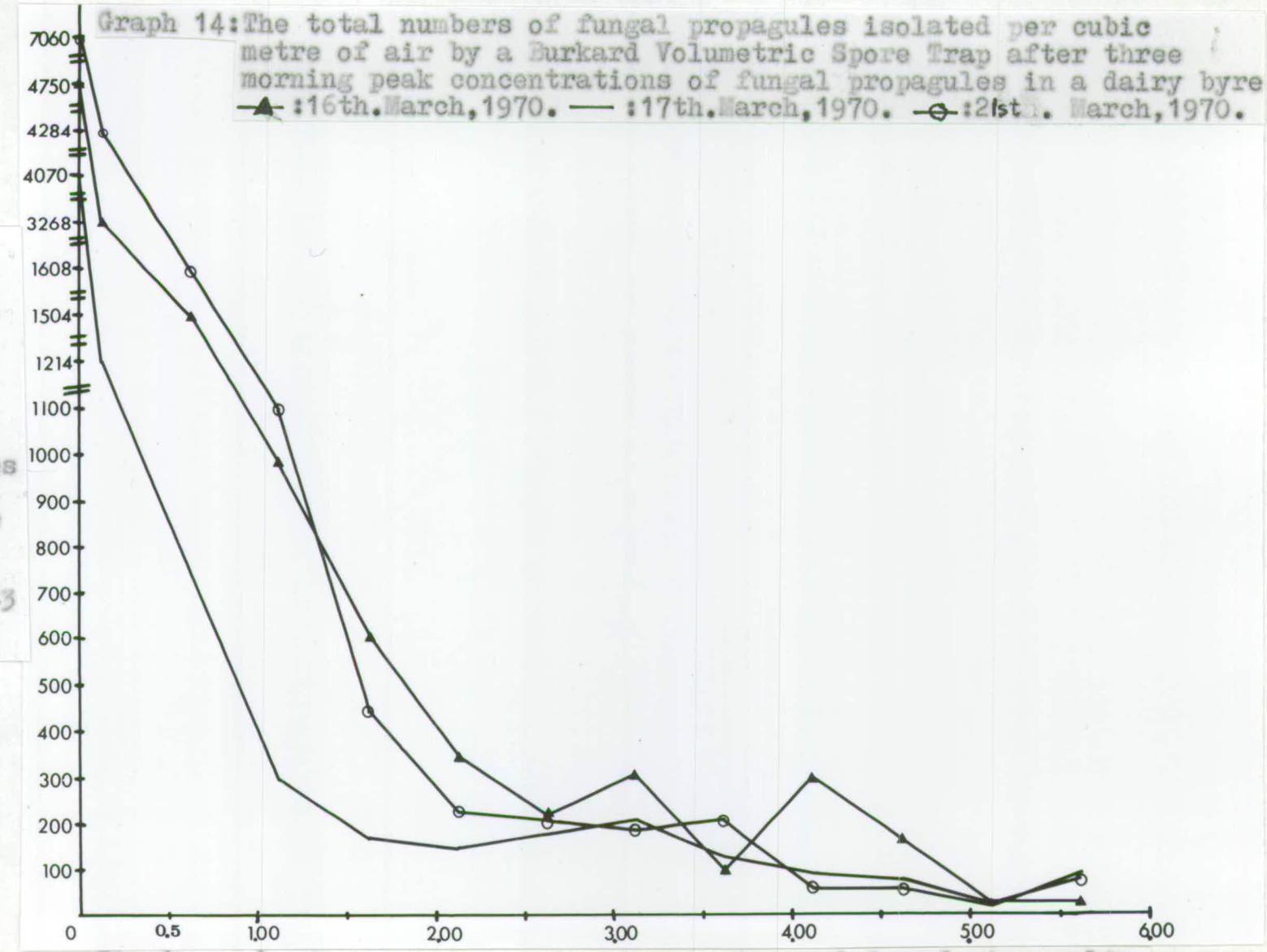
The numbers of fungal propagules counted after both morning and evening peak values at times detailed in Graphs 3 and 4, were added together and the



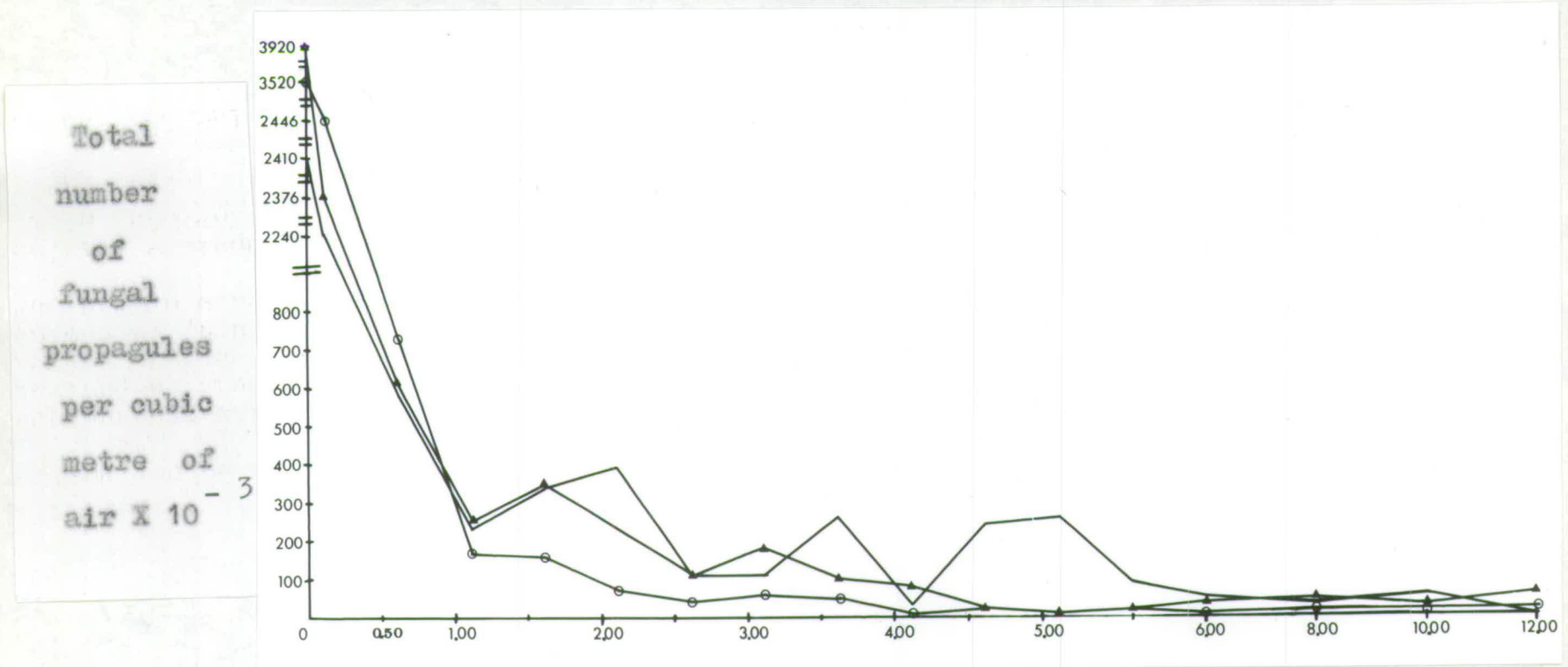
Graph 13: The number of spores of the Aspergillus/Penicillium type isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three evening peak concentrations of fungal propagules in a dairy byre.

—▲— :16th.March,1970 —— :17th.March,1970. —o— :20th.March,1970.

Graph 14: The total numbers of fungal propagules isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three morning peak concentrations of fungal propagules in a dairy byre
▲—▲ :16th. March, 1970. —— :17th. March, 1970. o—o :21st. March, 1970.



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Graph 15: The total numbers of fungal propagules isolated per cubic metre of air by a Burkard Volumetric Spore Trap after three evening peak concentrations of fungal propagules in a dairy byre.
▲ :16th.March,1970. — :17th.March,1970. -o-: 20th.March,1970.

proportions of different types of fungal propagules were expressed as a percentage of that total. In Table 3 it can be seen that the proportion of spores of Mucorales type and of Aspergillus/Penicillium-type after peak concentration in the morning were 48% and 30% respectively. In the evening, however, the Aspergillus/Penicillium-type spores were more common, making up 41% of the total while 43% were spores of the Mucorales. The proportion of spores of Cladosporium spp. was higher in the morning than in the evening (14% and 9% respectively). Other fungal propagules made up 8% of the morning total and 7% of that in the evening. Of these miscellaneous propagules, the largest percentage in the morning consisted of spores of Humicola spp. and in the evening, of hyphal fragments (Table 3).

The decrease in concentration of fungal propagules with time from three morning and three evening peak concentrations.

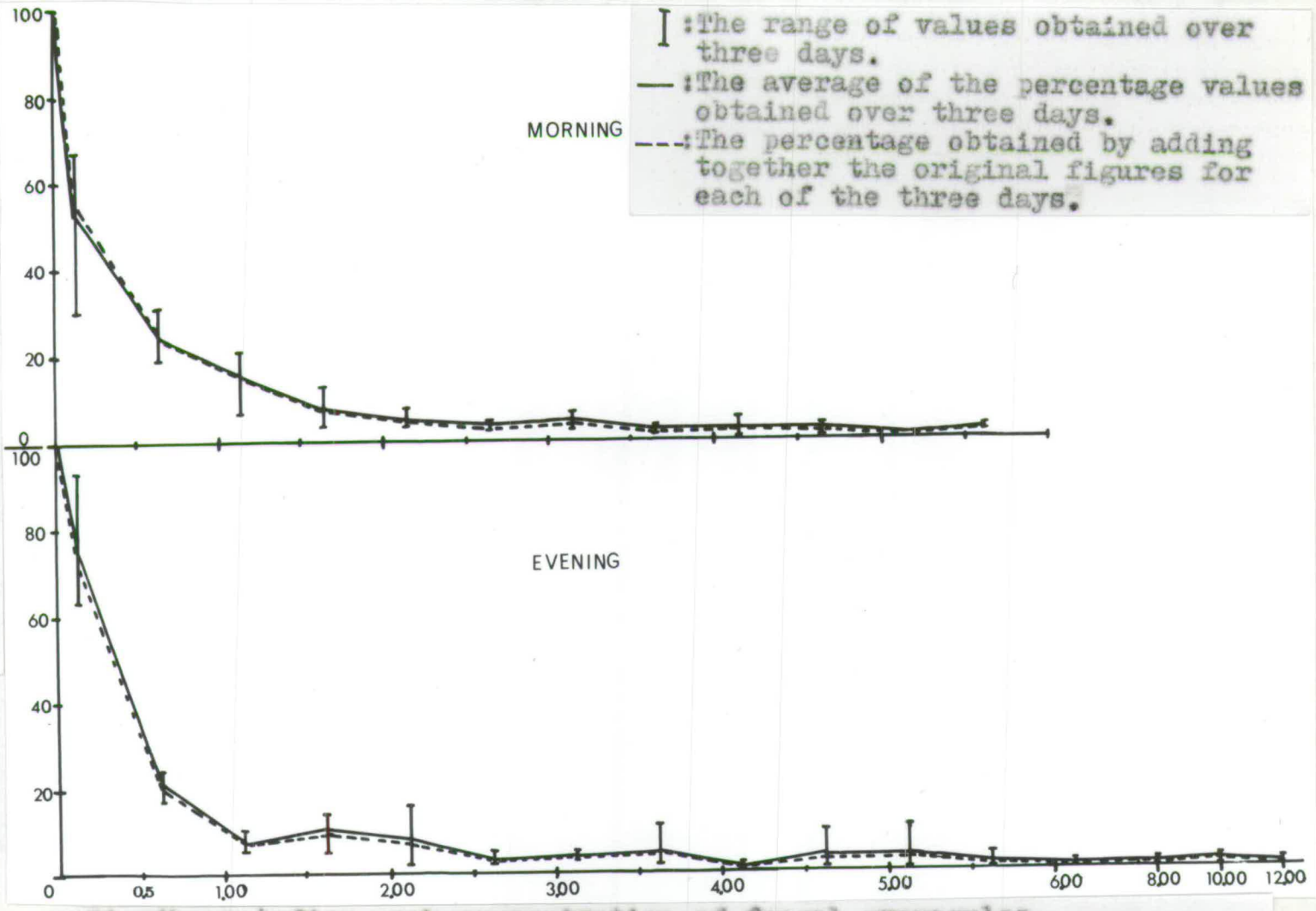
Graph 16 illustrates the difference between the decrease in spore concentration from peak morning and that from peak evening values, expressed as percentages of the original peak values. The range of percentages of the individual daily peak values at particular times over the three days are shown, together with the average of these three results. After initial wide ranges of percentages at 0-15 minutes after peak concentration, the ranges became narrower for both morning and evening. The morning peak concentration decreased to 53% within 0-15 minutes but the evening value decreased to only 75% within the same time interval. Similar results, of decreases to 55% and 72% of peak morning and evening values, respectively, were obtained by expressing the total of the three individual counts for peak concentration as 100%. The three values obtained at times specified in Graph 16 were added together and expressed as a percentage of the total three day peak value. The two methods of expressing the decrease in concentration gave almost identical results from 30 minutes after peak concentration, onwards, for both morning and evening values. After 30 minutes the results of 25% and 21% of the original concentration for morning and

Type of Fungal Propagule	Percentage of Total			
	Morning		Evening	
Spores of Mucorales type	48		43	
Spores of Aspergillus/Penicillium Type	30		41	
Spores of Cladosporium spp.	14		9	
'Others'	8		7	
"Others'	%TOTAL	%'OTHERS'	%TOTAL	%'OTHERS'
Hyphal Fragments	1.6	20	2.3	35
Spores of Humicola spp.	4.6	58	1.5	22
Spores of Epicoccum spp.	0.1	1	0.1	1.5
Sporangial heads of Mucorales	0.3	4	0.2	3
Spores of Alternaria spp.	<0.1	<1	0.1	1.5
Spores of Tricothecium roseum	0.10	1	0.4	6
Spores of other fungi	1.3	16	2.1	31

Table 3: The percentage of different types of fungal propagule after morning+ and evening* peak propagule concentration.

+: At alternate 15 minute intervals for up to 5 hours 30 minutes after peak concentration.

*: At alternate 15 minute intervals for up to 6 hours, and thereafter at 2-hourly intervals for up to 12 hours after peak concentration.



Graph 16: The decrease in the numbers of fungal propagules from morning and evening peak concentrations, expressed as a percentage of the value at peak concentration, over a period of three days

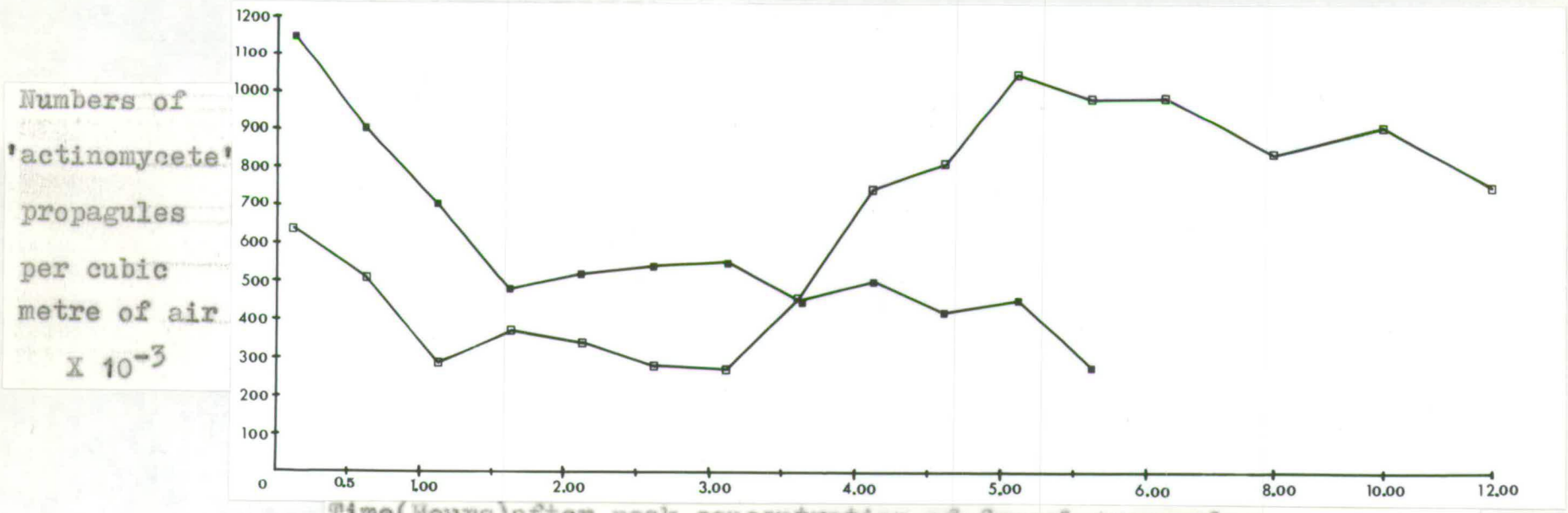
evening respectively indicate a situation similar to that shown in Graph 2, in which the numbers of fungal propagules decreased to 25% of the original value after 30 minutes.

iv) Distribution of Propagules of the dimensions of actinomycetes or bacteria after peak morning and evening concentration on three days.

After peak concentration of fungal propagules in the morning and evening the numbers of small refractile bodies of the dimensions of actinomycetes or bacteria were counted. The average numbers of these refractile bodies/m^3 over three days are shown in Graph 17 and demonstrate the difference between the numbers after peak morning concentrations and those after peak evening concentrations. There was a decrease in numbers/m^3 in the morning, from 1,150 to 280 over 5 hours 30 minutes. In the evening, the numbers fell from 640/m^3 to 270/m^3 over the first 3 hours, increased to a peak of 1,140/m^3 after 5 hours and fell to 750/m^3 (almost to the value 0-15 minutes after peak concentration of fungal propagules) after 12 hours.

Although there was considerable variation in the values obtained at particular times, the pattern of all three evening concentrations (Graph 19) was the same and showed a decrease for up to 3 hours to 3 hours 30 minutes after peak concentration followed by an increase, to a greater or lesser extent, before a second decrease 12 hours after peak concentration.

There was no consistent pattern in the morning values for the numbers of these refractile bodies/m^3 (Graph 18). On 16th March there was a considerable decrease between 0-15 minutes and 1 hour after peak concentration; on the 21st there was a decrease of the same order as that for the 16th but over a period of 5 hours 30 minutes after peak concentration. On the 17th, the value at 0-15 minutes was extremely low and there was an overall, slight increase in the numbers/m^3 during the 5 hours 30 minutes after peak concentration of fungal propagules.



Graph 17:The numbers of propagules of the dimensions of actinomycetes isolated per cubic metre of air from a dairy byre after morning and evening peak concentration of fungal propagules over three days.

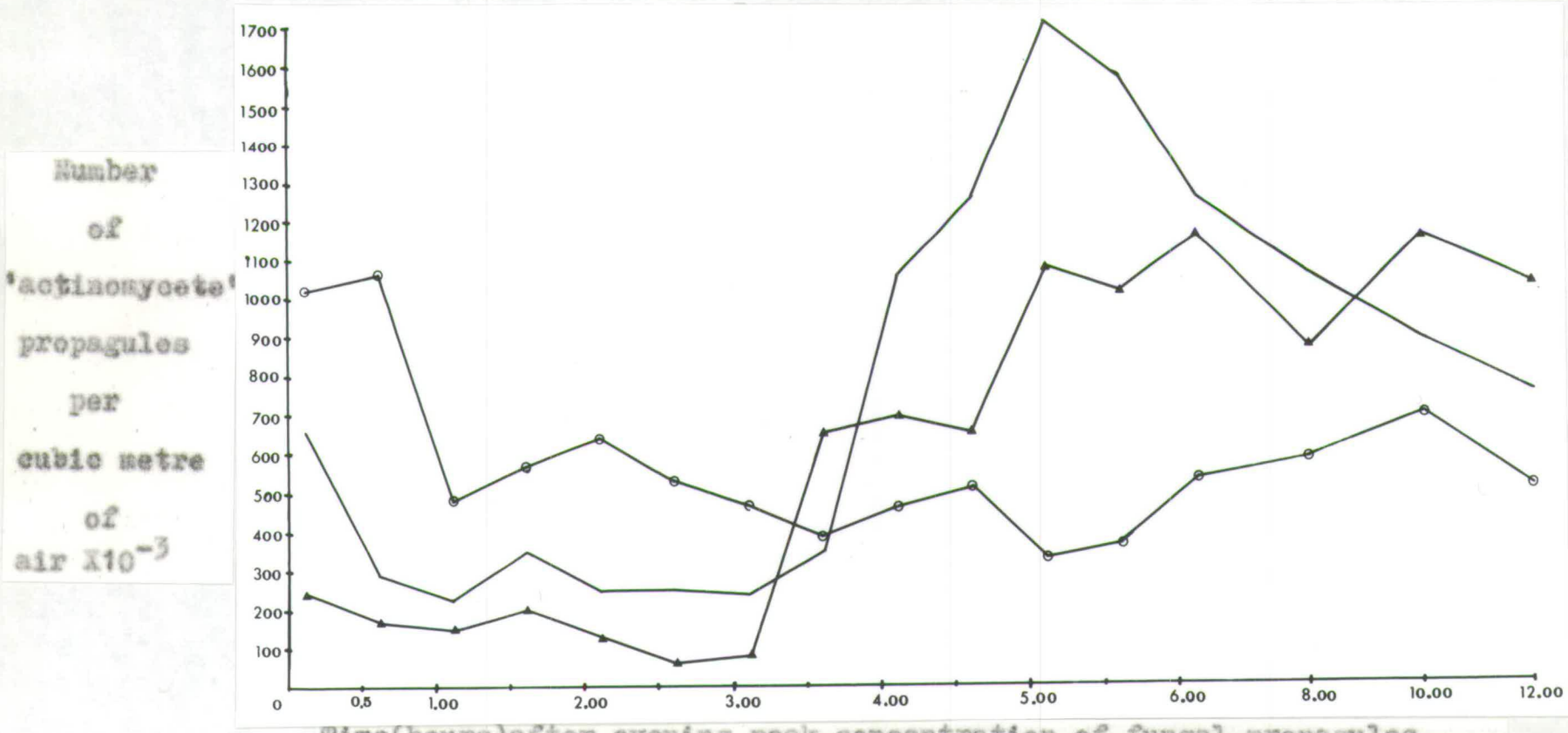
□ :after evening peak concentration

■ :after morning peak concentration

Graph 18: The numbers of propagules of the dimensions of actinomycetes isolated per cubic metre of air from a dairy byre after morning peak concentration of fungal propagules on three days.

▲ 16th.March, —— :17th.March, —O— :21st.March.





Graph 19: The numbers of propagules of the dimensions of actinomycetes isolated per cubic metre of air from a dairy byre after evening peak concentration of fungal propagules on three days.

▲ : 16th March, —— :17th.March, —O— : 20th.March.

2. Air Sampling by the Gravity Petri-Dish (G.P.D.) Technique.

a) In relation to activities of animal husbandry

Preliminary experiments on the culturing of viable airborne micro-organisms by the G.P.D. technique in 1969, indicated a correlation between activities of animal husbandry and the numbers of organisms isolated from the atmosphere of the byre. Figure 13 illustrates the numbers of colonies of microorganisms isolated on gravity Petri-dishes containing 2% malt agar and exposed for 2 minutes on March 21st, 1969. After the feeding of cattle with bruised barley and concentrate the numbers of microorganisms were considerably less than the numbers isolated 10 minutes after unbaling of hay. This is in agreement with the results from the Burkard volumetric spore-trap (Graph 1), in which the equivalent times are 07.00 hr. and 08.00 hr., respectively.

Average results for 6 days in 1970 and 3 days in 1971 (Table 4) at times specified in Materials and Methods, (2), show that this was a general trend. Large numbers of colonies of microorganisms grown on malt agar at 26°C and of actinomycetes grown on nutrient agar plus cycloheximide at 46°C are evident after foddering with hay and bedding with barley straw. Values were comparatively lower after feeding of barley and even fewer colonies were recovered between foddering times and in the late evening, approximately 4 hours after work had stopped in the byre.

The average numbers of fungi, isolated on 2% malt agar incubated at 26°C, over the period of three days in March 1971 were of the same order as those for the six days in 1970 but the numbers of total mesophilic organisms for 1971 were much lower at peak times than those for 1970. It therefore appears that a larger proportion of the mesophilic air-borne micro-organisms in 1971 were fungi compared with the proportion in 1970. Counts of mesophilic bacteria and actinomycetes were not made in 1970 but actinomycete counts in 1971 yielded only two settle- gravity plates with mesophilic actinomycetes.

The numbers of thermophilic actinomycetes in 1971 were less than 50%

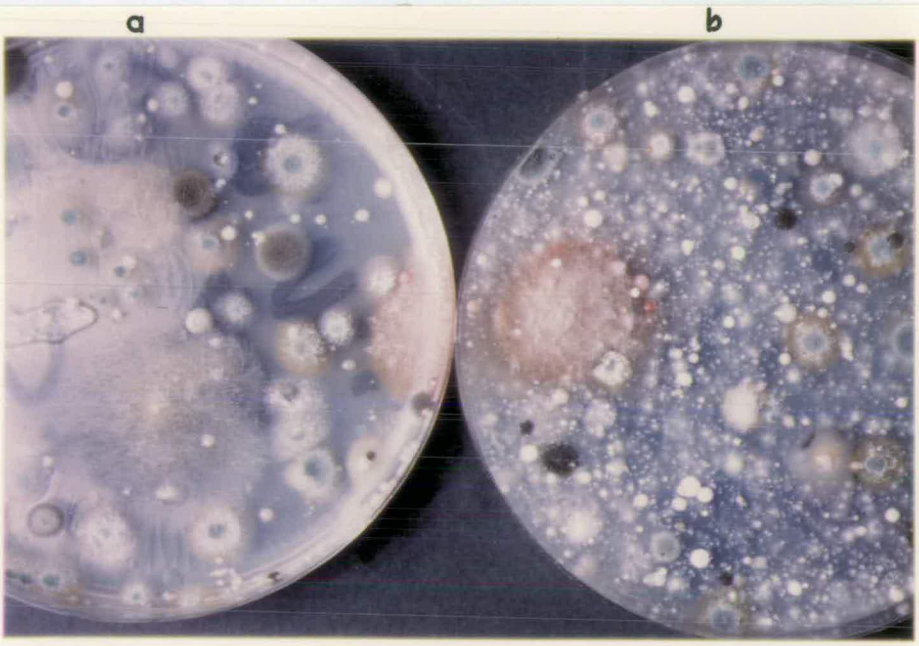


Figure 13: The airborne microflora of a dairy byre collected on gravity Petri dishes containing 2% malt agar incubated at 26°C.
a) after cattle had been fed on bruised grain and concentrate.
b) Ten minutes after unbaling of hay.

NO.	AVERAGE TIME	ASSOCIATION WITH WORKING SCHEDULE IN BYRE	1970			1971		
			ACTS N+C 46°	TOTAL MALT 26°	FUNGI MALT 26°	ACTS N+C 46°	TOTAL MALT 26°	FUNGI MALT 26°
1	07.30	After barley fed to cattle	12	108	103	7	133	108
2a	08.00	After hay fed to cattle	23	520	245	8	288	235
2b	08.10	10mins.after hay fed to cattle	31	531	269	10	301	298
3	14.05	Between feeding times	11	180	84	7	129	65
4	14.40	After barley fed to cattle	13	276	143	4	238	154
5a	17.20	After hay fed to cattle	30	620	268	12	284	227
5b	17.30	10mins.after hay fed to cattle	34	640	279	15	290	277
6	21.20	4 hr. after hay fed to cattle	7	93	59	5	80	72

Table 4: The numbers of organisms isolated by the gravity Petri-dish technique from a dairy byre in relation to the activities of animal husbandry during six days in March 1970 and three days in March 1971.

Acts N+C 46°C : The numbers of thermophilic actinomycetes isolated on 2% agar containing ½-strength nutrient broth plus 500 mg. cycloheximide/1. and incubated at 46°C.

Total Malt 26°C : The total numbers of mesophilic organisms isolated on 2% malt agar incubated at 26°C.

Fungi Malt 26°C : The numbers of mesophilic fungi isolated on 2% malt agar incubated at 26°C.

those of 1970 at peak values and did not show the approximately three-fold increase between off-peak and peak times, evident in 1970. The 1971 results did, however, show a slight increase after barley feeding and unbaling of hay and straw.

b) In relation to the decrease in fungal propagule concentration with time after unbaling of hay and straw.

Graph 2 shows the results obtained in 1971, using the G.P.D. technique to estimate the decrease in the numbers of viable fungal propagules with time from peak concentration in the dairy byre. Peak concentration time was taken as being 10 minutes after unbaling of hay and straw. The results subsequently obtained at 30 minute and 60 minute intervals were expressed as a percentage of the peak concentration. The results for the first three hours are an average of three figures and for the next three hourly intervals, an average of two figures. The range of percentages obtained for each time is also shown in Graph 2. There is a narrow range at 30 minutes, 1 hour and 6 hours after peak concentration but a wide range of values at the intervening times, and the ranges overlap one another. Although the validity of one individual result, in this central area of the graph, may be dubious, there is a general downward trend in the numbers of viable fungal propagules, indicating the expected decrease in concentration of propagules with time. At the very high or very low concentrations of propagules at either end of the time interval studied, the indication of a decrease in the numbers of microorganisms with time is more marked.

From these results, the numbers of viable fungal propagules had dropped to 85% in 30 minutes and to 68% in 1 hour. After 4 hours the concentration decreased to 26% and after 6 hours to 11% of the original. In 1970, over a period of 6 days, the decrease from the peak numbers of fungal propagules was to an average figure of 22% after 4 hours and was within the range obtained in 1971, for this time. This figure was also, however, within the range of values obtained at 2 hours, 2 hours 30 minutes and 5 hours after peak concentration.

3. Air Sampling by the Andersen Sampler, in Conjunction with the Gravity Petri-Dish (G.P.D.) Technique.

Results obtained by using the Andersen Sampler in conjunction with the G.P.D. technique on March 22nd, 1972 are shown in Table 5. The number of colonies of mesophilic fungi isolated by the Andersen Sampler per m^3 of air, 10 minutes after unbaling of hay and straw was 2,504 x $10^3/m^3$ compared with a count of 904 from the G.P.D. The numbers of thermophilic actinomycetes on N+C and of thermophilic actinomycetes plus fungi on G.A., both incubated at 46°C, were 136 x $10^3/m^3$ and 358 x $10^3/m^3$, respectively. The equivalent G.P.D. results were 48 per plate for both N+C and G.A. These results indicate a predominance of fungal propagules in the viable airborne microorganisms in the atmosphere of the byre.

The results for the decrease in concentration of mesophilic fungal colonies isolated by the Andersen Sampler are incorporated into Graph 2 and exhibited a rapid drop after 30 minutes to 29% of the original concentration. There was a further slight decrease to 24% after 1 hour 30 minutes and the value fell to 0.6% of the original after 6 hours. These results are of the same order as those found in the decrease in numbers of fungal propagules obtained using the Burkard volumetric spore trap, which was to 0.8% of the original after 6 hours (Graph 2).

G.P.D. results in Table 5 gave a percentage decrease in numbers of fungal propagules isolated at 26°C, similar to those obtained in 1971, for equivalent times (Graph 2). The numbers of colonies on malt agar were much higher at peak concentration than those for 1970 or 1971.

The Andersen Sampler results showed a considerable decrease in the numbers of colonies isolated on N+C and G.A. incubated at 46°C, with time, but this decrease was only partially reflected by the G.P.D. results which remained at 44% and 31% of the original values after 6 hours.

Species of fungi and actinomycetes isolated by the gravity Petri-dish technique and by the Andersen Sampler

Figure 14 contains a list of fungal species isolated by the G.P.D.

TIME	NUMBER OF VIABLE PROPAGULES/M^3 OF AIR X 10^{-3} OBTAINED BY THE ANDERSEN SAMPLER			NUMBER OF VIABLE PROPAGULES PER PLATE; ISOLATED BY THE GRAVITY PETRI-DISH TECHNIQUE		
	FUNGI MALT 26°C	ACTS N+C 46°C	FUNGI + ACTS GA 46°C	FUNGI MALT 26°C	ACTS N+C 46°C	FUNGI + ACTS GA 46°C
PEAK CONCENTRATION (P.C.)	2,504	136	358	904	48	48
P.C. + 30 min.	726	24	37	864	21	36
P.C.+1hr.30min.	589	22	16	272	25	10
P.C. + 6 hr.	14	14	4	140	21	15
	PERCENTAGE OF PEAK CONCENTRATION					
P.C. + 30 min.	29	18	10	96	44	75
P.C.+ 1hr.30 min.	24	16	4	30	52	21
P.C. + 6 hr.	0.6	10	1	15	44	31

Table 5 : Results obtained by the Andersen Sampler and gravity Petri-dish technique for the numbers of viable propagules in the air of a dairy byre at peak evening concentration of propagules and at intervals of time after peak concentration. The decrease in the numbers of particular propagule types is expressed as a percentage of their value at peak concentration.

Fungi Malt 26°C : The numbers of fungal colonies isolated on 2% malt agar incubated at 26°C.

Acts N+C 46°C : The numbers of colonies of actinomycetes isolated on 2% agar containing ½-strength nutrient broth plus 500 mg. cycloheximide/1. and incubated at 46°C.

Fungi + Acts, GA 46°C : The numbers of colonies of fungi and actinomycetes isolated on glycerol asparaginate agar incubated at 46°C.

	1971	1972	
Mesophilic Fungi	G.P.D.	G.P.D.	A.S.
Acremoniella atra	+		
Alternaria sp.	+		
Aspergillus candidus			+
Aspergillus cervinus	+		
Aspergillus fumigatus	+	+	+
Aspergillus glaucus	+	+	+
Aspergillus nidulans	+		
Aspergillus niger	+		
Aspergillus ornatus	+		+
Aspergillus ustus	+		
Aspergillus versicolor	+		+
Aureobasidium pullulans	+	+	+
Botrytis cinerea			+
Cephalosporium sp.	+		
Chaetomium sp.	+	+	+
Chrysosporium sp.	+		
Cladosporium sp.		+	+
Epicoccum sp.	+		
Fusarium sp.	+		
Geotrichum sp.		+	+
Gliomastix sp.	+		
Mucorales	+	+	+
Mycelium sterilis	+		
Penicillium spp.	+	+	+
Scopulariopsis spp.	+		
Trichoderma viride	+	+	+
Verticillium sp.	+		+
Yeasts	+	+	+
Unidentified	+		
Thermophilic Fungi			
Aspergillus fumigatus	+	+	+
Humicola spp.	+	+	+
Mucorales	+	+	+
Unidentified			+

Figure 14: Mesophilic and thermophilic fungal species isolated by the gravity Petri-dish (G.P.D.) technique and the Andersen Sampler (A.S.) at time intervals after peak evening concentration of fungal propagules during one day in March 1972 and by the G.P.D. technique at 8 time intervals during each of 3 days in March 1971.

technique and by using the Andersen Sampler on March 22nd, 1972 at times specified in Table 5. Species isolated by the G.P.D. technique during three days in 1971, at times specified in Table 4 are also shown. The larger numbers of mesophilic fungal species isolated in 1971 (24 species), compared with 15 species isolated in 1972, may be a reflection of the number of times samples were taken. The larger number of colonies per plate in 1972 may, however, have caused less common species to be overgrown or inhibited.

The number of species (15) isolated by the Andersen Sampler run for 10 seconds at an air-flow of 25 litres per minute was greater than the number (10 species) isolated by G.P.D.s exposed for 2 minutes.

Unidentified mesophilic fungi, bacteria and actinomycetes were also recorded in 1971 and 1972.

Thermophilic fungi, bacteria and actinomycetes were recorded in both years. Species of thermophilic fungi isolated were limited to the genera Aspergillus and Humicola and the Order Mucorales. Thermophilic actinomycetes from the Andersen Sampler plates in 1972 consisted of brown, white, blue, yellow and small, more-or-less colourless colonies and included species of Streptomyces and the species Micropolyspora faeni and Thermoactinomyces vulgaris.

4. A comparison of results obtained by the gravity Petri-dish technique and by using the Burkard volumetric spore trap or the Andersen Sampler.

1. Numbers of fungal propagules present in a dairy byre.

The ratio of the highest to the lowest value obtained from the number of fungal propagules/m^3 on a twenty-four hour spore trace from the Burkard volumetric spore trap was 1,632 : 1. Over six days in 1970, the ratio of the highest to the lowest G.P.D. result was 20 : 1. Between peak evening concentration and 6 hours after peak concentration the ratio for the spore trap trace results was 127 : 1 and for the Andersen Sampler was 179 : 1. The G.P.D. results, however, gave a ratio of only 7 : 1, demonstrating the

lack of sensitivity of this method in monitoring concentrations of fungal propagules.

The percentage decrease in the numbers of viable fungal colonies isolated by the Andersen Sampler within 30 minutes from a peak concentration value was of the same order as that obtained from the Burkard spore trap trace. Although the counts for propagules isolated at peak concentrations on the trace are probably inaccurate, this would not alter the order of difference of concentration considerably. Gravity Petri-dish results showed a consistently less sharp decrease with time (Graph 2).

2. Numbers of actinomycetes present in a dairy byre

The ratio of the highest value to the lowest value for numbers of thermophilic actinomycetes isolated on N+C by the Andersen Sampler is 10 : 1 and for the equivalent G.P.D. results is 2 : 1, for March 22nd, 1972. For six days in 1970, the equivalent ratio for G.P.D.'s is 4 : 1. There were smaller numbers of colonies isolated and a smaller range of values for numbers of thermophilic actinomycetes than for fungi.

As many propagules of the dimensions of actinomycetes or bacteria were probably hidden at times of high fungal propagule concentration, results from the Burkard volumetric spore trap at these times cannot be considered to be accurate. At low concentrations of fungal propagules, relatively high counts for actinomycete/bacterial type propagules were recorded. This method is of little value in assessing numbers of actinomycetes as it cannot differentiate between propagules of actinomycetes and bacteria. It does indicate, however, that relatively large numbers of propagules of dimensions capable of penetration to the alveoli of the lungs are present in the air of the byre several hours after the concentrations of other propagules have become negligible.

Conclusions

1. There are yearly variationsin the macroscopic appearance of spore traces obtained by using the Burkard volumetric spore trap. These variations reflect the microbiology of the products used for feeding, foddering or bedding cattle.

2. The numbers of fungal propagules counted on the regions of peak density of the trace have, in general, a qualitative correlation with the absorbance of the equivalent region measured on the Joyce Chromoscan. Anomolies between results obtained by the two methods are probably due to the fact that these two methods measure different parameters. One measures the numbers of fungal propagules; the other, the absorbance of light by such propagules. Small numbers of pigmented fungal propagules such as spores of Alternaria spp. or Epicoccum spp. are capable of absorbing a larger amount of light than large numbers of small, less strongly pigmented spores such as those of the majority of the Aspergilli and Penicillia.

3. Microscopic examination of traces from the Burkard volumetric spore trap give an indication of the numbers of fungal spores and hyphal fragments present in the atmosphere of the dairy byre at different times of the day. Variations in the numbers of fungal propagules/m^3 correlate with activities of animal husbandry, notably the unbaling of hay or straw which was associated with the highest counts on the trace.

4. Figures for one twenty-four hour period ranged from 3×10^3 to $4,897 \times 10^3$ fungal propagules/m^3 and realised an average of $264 \times 10^3/m^3$. The twenty-four hour period contained five distinctive time intervals; two time intervals of peak propagule concentration associated with feeding, foddering and bedding, one 'off-peak' time interval between feeding and foddering times with comparatively low counts and two off-peak time intervals with very low counts, in the late evening and early morning.

5. Detailed examination of spore types present for up to 5 hours - 30 minutes after 3 morning, and for up to 12 hours after 3 evening peak concentrations led to the following conclusions;

i) Initially, at peak concentration, there are higher values in the morning than in the evening, for the total numbers of fungal propagules and for the numbers of spores of Mucorales type, Aspergillus/Penicillium type and Cladosporium spp.

ii) There is a maintenance of concentration of larger spore types (those of Mucorales type and Cladosporium spp.) in the air after morning peak concentration while Aspergillus/Penicillium type spores predominate from 1 hour after peak evening concentration. Activity in the byre during the day (Figure 1) and the subsequent disturbance of the atmosphere probably accounts for the higher number of larger spores present at this time.

iii) Sporadic increases in the numbers of fungal propagules on traces after peak evening concentration are largely attributable to increases in the numbers of Aspergillus/Penicillium type spores. These increases are probably due to animal movement in the byre causing sufficient disturbance to make smaller spore types airborne.

iv) The numbers of Aspergillus/Penicillium type spores at a particular time is inversely proportional to the number of spores of the Mucorales type. This may be due to large spores masking the presence of smaller Aspergillus/Penicillium type spores on the trace but does not explain why the situation exists at very low spore concentrations.

v) The percentage decrease in numbers of fungal propagules during the first 0-15 minutes after peak concentration is highly variable over the three days studied. This initial variation may, in part, be due to the difficulties involved in counting propagules accurately at high concentrations. After 30 minutes the numbers of fungal propagules have dropped to an average of 25% and 21% of the original value for morning and evening respectively.

6. The numbers of colonies isolated by the gravity Petri-dish technique correlate, on average, with activities of animal husbandry but show considerable variation within any one sample time. This method of air sampling differentiates between very high and very low concentrations of viable propagules but does not accurately monitor intermediate concentrations.

7. Air-sampling by both the Burkard volumetric spore trap and the Andersen Sampler proved to be more sensitive in determining the concentration of fungal propagules than that by the gravity Petri-dish technique.

8. The Andersen Sampler isolated a greater number of different fungal species than gravity Petri-dishes at equivalent times. This is in agreement with the findings of Sayer et al., 1969.

9. The Andersen Sampler is more efficient than the gravity Petri-dish technique or the Burkard volumetric spore trap in enumerating the numbers of actinomycetes in the air.

10. The Burkard volumetric spore trap is of little value in assessing numbers of actinomycetes at times of high concentration of fungal propagules. It does indicate that relatively large numbers of propagules of dimensions capable of penetration to the alveoli of the lungs are present in the air of the byre, several hours after the concentrations of other propagules have dropped.

SECTION II

THE MICROBIOLOGY OF FARM ENVIRONMENTS IN SELECTED AREAS OF SCOTLAND WITH REFERENCE TO THE PREVALENCE OF FARMER'S LUNG DISEASE.

The Microbiology of Farm Environments in Selected Areas of Scotland with Reference to the Prevalence of Farmer's Lung Disease.

A pilot survey, to establish the prevalence of farmer's lung disease in selected areas of Scotland, was carried out between July, 1970 and January, 1971 (Grant et al., 1972 : Appendix E). The prevalence of the disease in the farming populations of Orkney, Ayrshire and East Lothian was found to be 86, 86 and 23 per 1000 respectively.

The materials and methods employed in surveying the 10% stratified samples (Grant et al., 1972) of the farming populations of Ayrshire and East Lothian between November, 1970 and January, 1971, are described below. A microbiological assessment of the air of farm environments and of the farm products associated with the farms surveyed is also described. In July, 1970, during the survey of the total farming population of Orkney, by a clinical team, a small number of samples of hay, grain and dust was collected and stored at $4^{o}C$. These samples were later subjected to the same microbiological analysis as those from Ayrshire and East Lothian.

Materials and Methods.

1. Respiratory disease in relation to location and employment.

A questionnaire was used to record the type and size of each farm and the methods employed in the management of cereal and forage crops. A second questionnaire, modified from that relating to respiratory disease according to the MRC, 1966, was completed for each farmer, farmer's wife and farm worker actively involved in farm work, in order to establish whether any had suffered from symptoms of farmer's lung disease within the previous five years and if so, whether these were associated with particular farming activities. Suspected cases were referred to hospitals for more detailed examination and diagnosis by clinical personnel. Samples of sera obtained from these suspected cases were tested against farmer's lung hay (F.L.H.) antigen by the routine serological testing service at the Western General Hospital, Edinburgh.

2. Sputum sampling.

Early morning samples of sputum, collected in sterile universal containers, were sent in by a small number of members of the farming populations of Ayrshire and East Lothian. Immediately on arrival the sample was divided as equally as possible between plates of 2% malt and glycerol-asparaginate agars (Appendix A), subsequently incubated at 26°C and 46°C respectively.

3. The microflora of the air of farm environments.

At each Ayrshire (Ayr) and E. Lothian (E.L.) farm, two 9 cm polystyrene gravity Petri-dishes were each exposed for a period of 2 minutes in regions where there was human exposure to organic dusts from animal foodstuffs, fodder and bedding materials. Typical sites were dairy byres, cattle courts, and pig sheds. As market gardens were also included in the survey, gravity Petri-dishes were exposed in greenhouses and outhouses. One of each set of plates contained 2% malt agar and was incubated at 26°C; the other, incubated at 46°C, contained glycerol-asparaginate agar. The number of colonies of fungi and actinomycetes cultured on the plates was counted after 3 days and fungi isolated were identified after 10 - 14 days. (Appendix D)

4. The microflora of randomly selected samples from farm environments.

From all farms in Ayr and E.L., samples of the following were collected when available in sterile, weighed, 25 ml., polystyrene, universal containers:

Hay, straw, grain, sawdust (when used for bedding animals)

silage and dusts off grain-bruising machines or hammer-mills.

Hay samples were designated 'good' or 'bad' on the basis of their degree of discoloration and superficial moulding. The samples were taken from the centres of bales by means of forceps previously sterilised by dipping in ethanol. Sub-surface grain was collected from silos, bins and polythene or jute bags using a sterile vial as a scoop to avoid undue superficial contamination. Materials were stored at 4°C until a random selection of each type was examined microbiologically. In order to assess the microflora

of samples in both quantitative and qualitative terms, the apparatus shown in Fig. 15 was employed. Each sample was shaken vigorously for 30 seconds on a Griffin flask shaker and then had a standard quantity of air (5 cc) blown over it by depressing the plunger. The microflora released during successive samplings was collected on the following media in 9 cn Petri-dishes:-

i) one plate of 2% malt agar subsequently incubated at 26°C.

ii) Two plates of glycerol-asparaginate agar; one plate incubated at 46°C, the other at 60°C.

Total counts of microorganisms and the numbers of colonies of fungi and actinomycetes were recorded at 3 days. The number of organisms per 10 g. sample was then calculated using the following correction:-

$$\text{Number of colonies per plate} \cdot \frac{10(25 - y)}{5} \cdot \frac{1}{x}$$

where y = number of ccs displaced by the material in a 25 cc. vial through which 5 cc. air was blown. (The density of materials examined was taken into account in this calculation).

x = weight of material in grammes.

Fungal colonies were identified (Appendix D) after 10 - 14 days incubation.

5. The relative occurrence of Micropolyspora faeni and Thermoactinomyces vulgaris in environmental samples showing high counts of thermophilic actinomycetes.

Environmental samples yielding high counts of thermophilic actinomycetes on the unselective glycerol-asparaginate agar were re-examined by direct plating and by air sampling (as in 4. above) onto 2% agar containing half strength nutrient broth (Oxoid) and 500 mg/litre cycloheximide, (Appendix A), to prevent fungal growth. After incubation at 46°C for three days the presence of colonies of M.faeni (Cross et al., 1968) and of

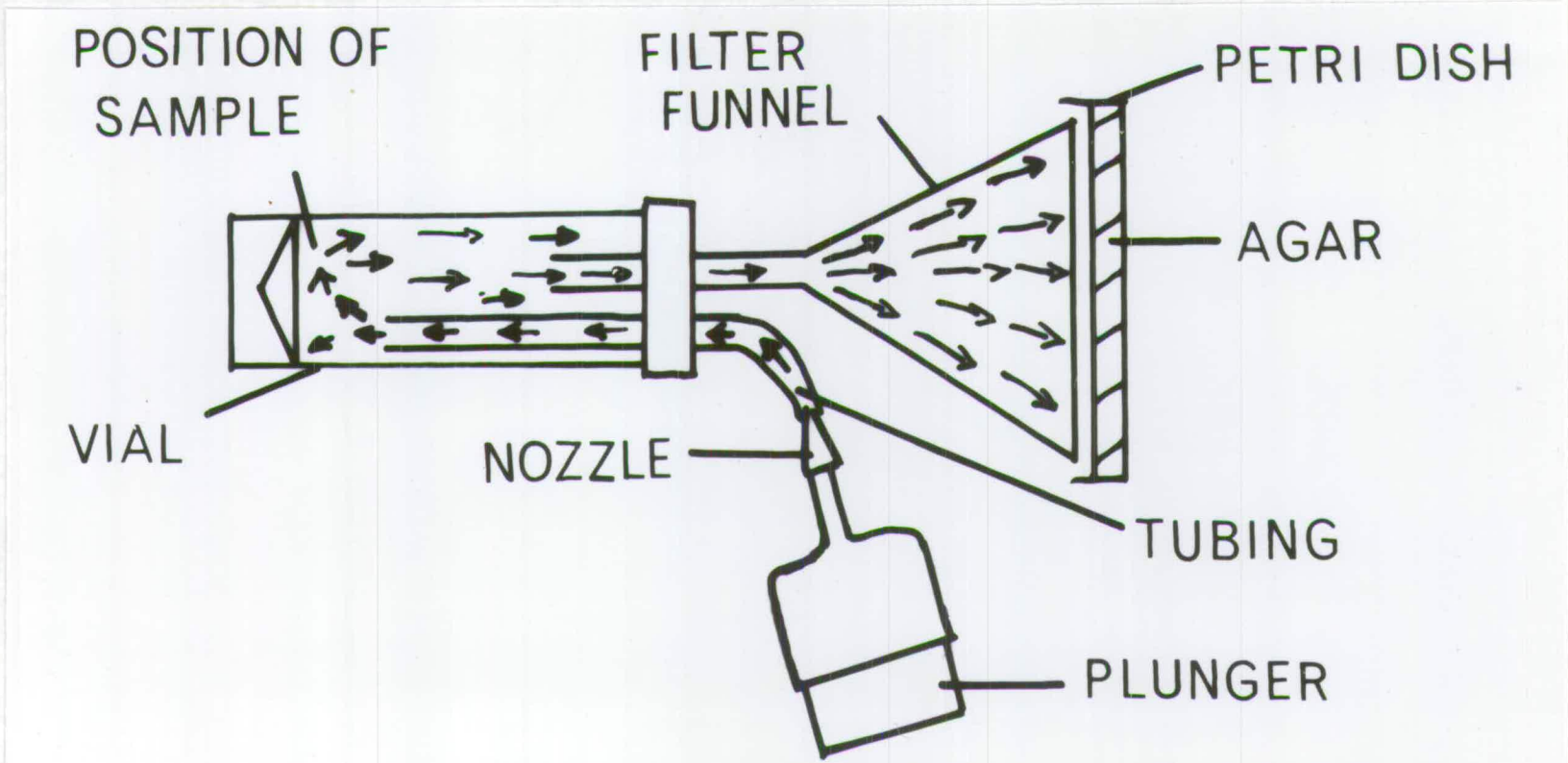


Figure 15:The apparatus employed to remove airborne propagules from samples of hay,straw,etc.

T. vulgaris (Cross, 1968) was noted.

Results

1. The number of personnel on farms and small holdings.

The distribution of the labour force on small, medium-sized and large farms determined the numbers of different types of environment surveyed (Grant, et al., 1972). Seventy-two percent of the farming population interviewed in Ayr was employed on dairy farms and sixty-eight percent of the E.L. population was employed on arable stock farms (Table 6).

2. Sputum.

Although the numbers of sputa sampled were small, there appeared to be a correlation between the incidence of culturable thermophilic actinomycetes and/or Aspergillus fumigatus and the prevalence of farmer's lung disease in the three regions (Table 7). Results obtained previously by Dr. W. Blyth, Department of Botany, University of Edinburgh, for samples of sputa from the Orkney farming population are included in this table.

3. Fungi and actinomycetes recovered by the gravity Petri-dish technique from the air of farm environments in Ayrshire and East Lothian

a) Total numbers

The numbers of each type of farm environment surveyed in Ayr and E.L. are recorded in Table 8. The average numbers of fungi and actinomycetes isolated at 26°C and 46°C were highly variable both within and between environmental groups in the two counties (Table 8). The numbers of fungi and actinomycetes isolated from outhouses were very low compared with those obtained from Ayr cattle-courts and dairy byres. The latter two groups produced considerably higher numbers of thermophilic organisms than any others, and were in sharp contrast to E.L. cattle-courts and dairy byres, where low figures were obtained. Ayr hay or straw sheds and E.L. pig-sheds yielded large numbers of thermophilic actinomycetes, the latter being the only E.L. environments with significantly larger values than their Ayr equivalents.

Type of farm	Number of the Farm Type Surveyed.	
	Ayrshire	East Lothian
Dairy	52(106)*	4(5)
Arable without stock	0	8(16)
Arable with stock	16(23)	41(101)
Stock	4(5)	2(3)
Market garden	8(13)	16(21)
Poultry	1(1)	1(2)
Totals	81(148)	72(148)

Table 6. Distribution of personnel (*) on the different types of farm surveyed in Ayrshire and East Lothian.

Region	Thermophilic Actinomycetes	Aspergillus fumigatus	Prevalence of farmer's lung disease/1000
Orkney	8/11	8/11	86
Ayrshire	7/8	8/8	86
East Lothian	4/11	6/11	23

Table 7. The incidence of colonies of thermophilic actinomycetes and of Aspergillus fumigatus cultured from sputa obtained from farming personnel in Ayrshire and East Lothian.

Numerator = number of positive samples.

Denominator = total number of samples.

Type of environment	Numbers in		Fungi				Actinomycetes			
	Ayr	E.L.	26°C		46°C		26°C		46°C	
			A.	E.L.	A.	E.L.	A.	E.L.	A.	E.L.
1. Outhouse (neither livestock nor plants)	1	7	7.0	7.2	0.0	0.1	0.0	0.3	1.0	0.0
2. Greenhouse	8	8	75.0	38.0	0.1	0.3	0.3	0.0	0.9	1.4
3. Potato shed	-	3	-	14.6	-	0.0	-	0.0	-	1.6
4. Hay or straw shed	2	6	17.0	13.0	3.0	0.7	0.0	2.7	39.0	1.7
5. Grain shed or granary	2	4	589.0	140.0	0.5	0.0	0.0	0.0	10.5	1.5
6. Pig shed	2	5	217.0	72.0	12.5	2.6	0.0	0.0	12.0	64.0
7. Hen house	2	1	665.0	500.0	2.5	0.0	0.0	0.0	8.0	8.0
8. Cattle court	5	22	287.0	283.0	117.0	5.0	4.0	0.2	103.0	3.7
9. Dairy byre	48	4	146.0	15.0	61.0	2.0	5.3	0.0	42.0	1.2
10. Non-dairy byre	5	-	132.0	-	111.0	-	9.0	-	14.0	-
11. Milking parlour	3	-	10.0	-	0.0	-	1.0	-	2.0	-

Table 8. The frequency of different types of farm environment sampled by the gravity Petri-dish technique in Ayrshire(Ayr) and East Lothian (E.L.). The average numbers of colonies of fungi and actinomycetes isolated from these environments are recorded from:-

'26°C' : 2% Malt agar plates incubated at 26°C.

'46°C' : Glycerol asparaginate plates incubated at 46°C.

The numbers of organisms isolated from Ayr milking-parlours were very low compared with Ayr dairy byres. The highest numbers of mesophilic fungi were recorded from hen-houses in both counties, and grain sheds in Ayr.

b) Groups and species

Mesophilic fungi, isolated at 26°C and thermophilic and thermotolerant fungi isolated at 46°C and 60°C are listed in Table 9. The distribution and percentage frequency of isolation of these fungal species and of mesophilic and thermophilic actinomycetes in different environments is shown in Table 10.

Alternaria sp., Aspergillus spp., Aureobasidium pullulans, Cladosporium sp., Penicillium spp., genera of the Mucorales and yeasts were widely distributed among the different environments.

Mesophilic actinomycetes, Aspergillus fumigatus, Botrytis cinerea, Epicoccum sp., Fusarium sp. and Mycelia sterilia were less common. The remaining mesophilic fungi were isolated only occasionally.

Thermophilic actinomycetes were present in all types of environment except E.L. outhouses. Thermophilic and thermotolerant fungi were most common in cattle-courts and dairy and non-dairy byres, but were rarely isolated from other environments.

The distribution of fungal species from E.L. and from Ayr greenhouses may be compared, as 8 greenhouses were sampled in each county. The same species were most frequently isolated in both counties, i.e. Aspergillus spp. other than those of the A. fumigatus group, the A.fumigatus group Aureobasidium pullulans, Botrytis cinerea, Cladosporium sp., Penicillium spp. and yeasts. There were, therefore, negligible differences between greenhouses in the two counties regarding fungal species isolated, as well as their percentage distributions. A comparison can also be made between the two main environmental types, i.e. E.L. cattle courts and Ayr dairy byres. Both types of environment contained the same dominant fungal species.

Fungal Species or Groups	Occurrence in:-	
	Air	Farm products
MESOPHILES		
Absidia spp.	+	+
Alternaria spp.	+	+
Aspergillus candidus Group		+
Aspergillus flavus Group	+	+
Aspergillus flavipes Group		+
Aspergillus fumigatus Group	+	+
Aspergillus glaucus Group	+	+
Aspergillus nidulans Group	+	+
Aspergillus niger Group	+	+
Aspergillus ochraceus Group	+	
Aspergillus ornatus Group		+
Aspergillus restrictus Group		+
Aspergillus terreus Group	+	+
Aspergillus ustus Group		+
Aspergillus versicolor Group	+	+
Aspergillus wentii Group	+	+
Aspergillus spp. unidentified	+	+
Aureobasidium pullulans	+	+
Acremoniella atra		+
Allescheria boydii		+
Botrytis cinerea	+	
Cephalosporium spp	+	
Circinella spp	+	
Cladosporium spp	+	+
Cylindrocarpon spp	+	
Cylindrocladium spp		+
Epicoccum spp	+	+
Fusarium spp	+	+
Gliocladium spp		+
Gliomastix spp		+
Mucor spp	+	+
Mycelium sterilis	+	+
Paecilomyces spp		+
Penicillium spp	+	+
Phoma spp	+	
Piptocephalis spp	+	
Rhizopus spp	+	+
Scopulariopsis spp	+	+
Torula spp	+	
Trichoderma viride	+	+
Trichothecium roseum	+	+
Verticillium spp	+	+
Yeasts	+	+
Unidentified	+	+
THERMOPHILES		
Aspergillus fumigatus Group	+	+
Humicola spp	+	+
Mucorales	+	+
Talaromyces duponti		+
Thermoascus aurantiacus		+
Unidentified	+	+

Table 9: The mesophilic, thermotolerant and thermophilic fungal species and groups isolated from farm products from East Lothian, Ayrshire and Orkney, and those isolated from the air of farm environments in the first two areas by the gravity Petri-dish technique.

MICROORGANISMS	ENVIRONMENTS																			
	1		2		3	4		5		6		7		8		9		10	11
	AYR	E.L.	AYR	E.L.	E.L.	AYR	E.L.	AYR	E.L.	AYR	E.L.	AYR	E.L.	AYR	E.L.	AYR	E.L.	AYR	AYR
MESOPHILIC FUNGI																			
Alternaria spp.		43		25	67		33				40			20	55	19	75	20	
Aspergillus fumigatus Group						50	17	50			20			20	14	31	50	80	
Other Aspergilli	100	14	63	38			83		25	50	20	50		40	64	58	100	60	33
Aureobasidium pullulans		28	38	25	33		17		50		80	50		20	55	20	50		
Botrytis cinerea		14	50	63			17	50	25								25		
Cephalosporium sp.			13													4			
Chaetomium sp.							17												
Cladosporium sp.	100	28	50	50	67		50	50						60	41	38	25	20	
Cylindrocarpon sp.											20								
Epicoccum sp.		14			33						20		100			2	25		
Fusarium spp.					33	50	17				20					8			
Mucorales		14	13	26		100	33	50	25	50	20	50	100	40	46	56	25	80	
Mycelium sterilis				13	33		17									2			33
Penicillium spp.		57	63	75	33	100	67	50	100	100	100	100	100	40	78	85	75	80	33
Phoma sp.											10								
Piptocephalis sp.				13							20								
Scopulariopsis sp.							33							20	5				
Torula sp.		28									20		100						
Trichoderma viride				13					25		20			20	14	10			
Trichothecium roseum				13					25							2	25		
Verticillium sp.											20								
Yeasts	100	71	50	50			17	100	50	100	60	100		20	41	40	75	40	67
MESOPHILIC ACTINOMYCETES		14	13				17							80	5	27		40	33
THERMOPHILIC FUNGI																			
Aspergillus fumigatus Group										50	20			40	18	38	50	80	
Humicola sp.		14	13			50	17							20	46	35	25	60	
Mucorales										50		50		40	32	23		40	
Unidentified														60		8		40	
THERMOPHILIC ACTINOMYCETES	100		25	25	33	50	50	100	50	100	80	100	100	80	68	81	50	60	67

Table 10: The percentage frequency of isolation of species or groups of fungi and actinomycetes from farm environments in Ayrshire (Ayr) and East Lothian (E.L.)

Environments are keyed in Table 8.

Alternaria sp. and Aureobasidium pullulans were considerably more common in E.L. cattle courts than in Ayr dairy byres, while A. fumigatus and mesophilic actinomycetes were more frequently isolated from Ayr dairy byres. All other species showed a similar distribution between the two counties or were isolated infrequently from one county only. Greenhouses, E.L. cattle courts and Ayr dairy byres had common dominant fungal species with two exceptions. Alternaria sp. was isolated frequently from E.L. cattle courts only and Botrytis cinerea was common in greenhouses, but was not isolated from either cattle courts or dairy byres.

4. Fungi and actinomycetes isolated from grain, silage, straw, hay and dust samples

a) Total numbers

The number of fungi and actinomycetes isolated from groups of samples of grain, silage, straw, hay and dust samples at 26°C, 46°C and 60°C were corrected to give the average numbers isolated per 10 g. of material. The results are recorded in Table 11.

i) Grain

Whole grain samples released low average numbers of organisms at all temperatures, but relatively large numbers of mesophilic fungi (502.0/10 g.) were recorded from Ayr barley. One Ayr barley, not included in the sample group but from an environment associated with a case of farmer's lung disease, yielded only 34.0 mesophilic fungi but 650.0 actinomycetes per 10g. at 60°C. Bruised barleys produced considerably more organisms at all temperatures than whole barleys. The numbers of thermotolerant and thermophilic organisms at 46°C and 60°C were much higher in Ayr than in E.L. samples.

ii) Silage

Ayr silage produced large numbers of mesophilic fungi and moderate numbers of actinomycetes at 60°C (22.4/10g.).

Type of Sample	Total Numbers	Nos. of Fungi/10g.			Nos. of Actinomycetes/10g.		
		26°C	46°C	60°C	26°C	46°C	60°C
Wheat (E.L.)	5	28.0	0	0	0	0.4	0.4
Oats (E.L.)	5	147.0	0	2.0	0	0	1.0
Oats (Ayr)	5	111.0	0.6	0.4	1.6	0	0
Barley (E.L.)	10	23.5	0.2	0	0.5	0	1.2
Barley (Ayr)	10	502.0	0	0	0	0	0
Bruised barley (E.L.)	10	219.0	0.7	0.4	6.0	17.0	4.0
Bruised barley (Ayr)	10	584.0	8.4	8.4	6.4	18.6	49.7
Silage (Ayr)	5	785.0	1.0	1.0	0	0	22.4
Oat straw (E.L.)	4	2725.0	0	0	9.0	0	0
Oat straw (Ayr)	5	896.0	0	0	164.0	0	o
Barley straw (E.L.)	10	403.0	0.9	2.3	7.4	0.4	0.7
Barley straw (Ayr)	16	964.0	9.0	0	1.0	33.0	487.0
Good hay (E.L.)	10	1272.9	0.6	0	0	5.6	0
Good hay (Ayr)	16	392.0	1.2	7.1	3.8	9.8	16.3
Bad hay (E.L.)	3	259.0	141.0	306.0	0	0	3.6
Bad hay (Ayr)	16	925.0	144.0	11.2	8.4	140.7	452.7
Hay (Orkney)	10	1003.0	216.0	192.0	1539.0	201.0	66.0
Sawdusts (Ayr)	5	215.0	3.0	0	0	0.8	3.0
Dusts (E.L.)	5	2984.0	34.2	33.4	0	44.2	20.4
Dusts (Ayr)	4	1318.0	111.0	246.0	0	240.7	159.5
Dusts (Orkney)	5	2263.0	42.0	31.0	0	102.0	48.0

Table 11: The average numbers of colonies of fungi and actinomycetes isolated from farm products from Ayrshire (Ayr), East Lothian (E.L.) and Orkney. Colonies were counted on Malt agar plates incubated at 26°C, and glycerol asparaginate plates incubated at 46°C and 60°C.

iii) Straw

Oat straw samples released large numbers of fungi at 26°C, E.L. samples producing the very high value of 2725.0/10 g. One E.L. sample not included in this group released 21,000 fungal propagules per 10 g. at this temperature. Ayr oat straw produced the largest number of mesophilic actinomycetes of all samples (164.0). No thermophilic organisms were recovered from oat straw, but were present in barley straw samples. Large numbers of thermophilic actinomycetes were released from Ayr barley straw at 46°C (33.0) and at 60°C (487.0/10g.) the latter being the highest number of thermophilic actinomycetes recovered from any group of samples at 60°C. Barley straw from both counties yielded high numbers of mesophilic fungi. One E.L. sample, included in the group of ten, contained few mesophilic fungi but was the only sample with thermophilic actinomycetes (4.4 at 46°C and 6.6 at 60°C).

iv) Hay

Ayr good hay samples contained fewer mesophilic fungi but more organisms in all other categories than their E.L. equivalents. Thermophilic fungi were isolated in greater numbers from E.L. bad hay samples than from any other hays, but actinomycetes were isolated at 60°C from one sample only, giving an average of 3.6. Ayr bad hay samples released more fungi at 26°C than their E.L. equivalents, and released more actinomycetes than any other hay samples, particularly at 46°C and 60°C.

v) Dusts and sawdusts

Ayr sawdusts released few fungi or actinomycetes. Dusts from both counties produced large numbers of mesophilic fungi. Thermophilic fungi and actinomycetes were recovered in relatively large numbers from dust samples obtained from both counties, Ayr dusts releasing many more than their E.L. equivalents. No mesophilic actinomycetes were isolated

from sawdust or dust samples.

vi) Orkney hay and dust samples

The Orkney hay collection consisted of a random sample, of which no representatives could be described as 'good' on visual examination. On average, they released large numbers of fungi and actinomycetes at all temperatures, producing as many thermophilic fungi as E.L. bad hay samples, more actinomycetes at 46°C than any other hays, but fewer actinomycetes at 60°C than Ayr bad hay samples.

Orkney dust samples had a similar distribution of fungi to that in E.L. dusts. No mesophilic actinomycetes were isolated, as in the case of other dust samples. The numbers of thermophilic actinomycetes were less than those for Ayr, but more than those for E.L. dusts.

b) Groups and species of fungi

Table 9 lists the fungal species and groups isolated from animal feeding stuffs, bedding and dust samples. A larger range of Aspergillus spp. but a narrower range of other fungal species was obtained from these materials than from gravity Petri-dishes exposed in farm environments.

c) Distribution of fungi and actinomycetes

The distribution of fungi and actinomycetes in feeding stuffs, bedding and dusts is expressed as the percentage of each sample type which contained a particular fungal or actinomycete species or group (Table 12). The species most commonly isolated were yeasts, Penicillium spp., Aspergillus spp., Trichoderma viride and members of the Mucorales. Thermotolerant Aspergillus fumigatus and thermophilic actinomycetes were also isolated frequently. Sixty percent or more samples of oats and oat straw contained Trichoderma viride. Aureobasidium pullulans was isolated from sixty percent of E.L. oat samples and from fifty percent of E.L. bruised barleys. Other fungal species or groups were isolated infrequently.

There was little difference in the species isolated from comparable

MICROORGANISMS	SAMPLES																			
	Wheat E.L.	Oats E.L.	Oats Ayr	Barley E.L.	Barley Ayr	Bruised Barley E.L.	Bruised Barley Ayr	Silage Ayr	Oat straw E.L.	Oat straw Ayr	Barley straw E.L.	Barley straw Ayr	Good Hay E.L.	Good Hay Ayr	Bad Hay E.L.	Bad Hay Ayr	Sawdusts Ayr	Dusts E.L.	Dusts Ayr	Hay 0	Dusts 0
MESOPHILIC FUNGI																					
Allescheria boydii																				10	
Alternaria sp				10					20	20	40	19									20
Acremoniella atra																					
Aspergillus fumigatus group					10		10	40	40	20	10	13		19	33	25	40			40	
Other Aspergilli				40	10	20	20	20	40	60	50	38	30	50	67	63	40	60	75	80	60
Aureobasidium pullulans		60	20			50	10		20		10	19				6	20				
Cladosporium spp				10					20		10	6						20			
Cylindrocladium sp			20				10													10	
Epicoccum sp											10	13				6					
Fusarium spp						20															
Gliocladium sp			40				10														
Gliomastix sp			20				20				10	19	20	25	33	56	20	20	50	40	20
Mucorales						10							10								
Mycelium sterilis						10						6									
Paecilomyces		40		90		50	100	40	60	40	70	81	90	69	100	81	80	100	75	40	60
Penicillium spp														19		13				20	
Scopulariopsis sp		100	80	10	10	50		20	60	60		13	20	13		38	20		50	40	40
Trichoderma viride													10								
Trichothecium roseum						10		40													
Verticillium sp	100	100	100	60	50	80	50	100	100	100	50	88	60	63	33	81	60	60	50	80	60
Yeasts									20				10	6		6					
Unidentified																					
MESOPHILIC ACTINOMYCETES			20	40		10	10		20	40	10	6		6		6				40	
THERMOPHILIC FUNGI																					
Aspergillus fumigatus group		20	40	10		10	10	20					10	13	33	31		40	100		
Humicola sp.							10					6		6		25		40	50	30	40
Mucorales				10										6	33	25		20	75		
Talaromyces duponti															33						
Thermoascus aurantiacus																					20
Unidentified THERMOPHILIC ACTINOMYCETES	20	20		20		30	20	20			10	31	10	6 38	33	56	40	20 60	100	60	80

TABLE 12: The percentage frequency of isolation of species or groups of fungi and actinomycetes from farm products in East Lothian (E.L.) Ayrshire (A) and Orkney (O).

samples in the two counties eg. E.L. and Ayr barley straw. With few exceptions this applied to all samples. The differences in the frequency of occurrence of those species between comparable groups were slight, e.g. good hays (Ayr) and bad hays (Ayr). The frequency of isolation of all species present in more than thirteen percent of these samples was greater from bad Ayr hays than from good Ayr hays. Thermophilic actinomycetes were isolated from fifty percent or more bad Ayr hays and dusts from both counties.

5. The incidence of farmer's lung disease and the frequency of isolation of thermophilic actinomycetes.

a) Total actinomycetes on glycerol asparaginate agar

The farm environments and materials containing the largest numbers of thermophilic actinomycetes are shown in Table 13, together with the numbers of associated cases of farmer's lung disease. The results obtained both from farm environments and samples of farm products may be misleading in that a small number of very high values within a sample group can produce a spuriously high average value. Similarly, a small sample group could give a very high percentage of positive counts, e.g. 2/2 being 100%. It is therefore, necessary to take both sets of results into consideration. As only one of the two Ayr or straw sheds yielded a high value for thermophilic actinomycets, this environmental group was not included in Table 13.

i) The microbiology of the air of Ayrshire and East Lothian farm environments.

The two types of Ayrshire environment containing the largest numbers of thermophilic actinomycetes were associated with eight out of thirteen cases of farmer's lung in Ayrshire; cattle courts were associated with two cases, dairy byres with six cases. Of the remaining five cases, three were associated with bulk grain handling at harvest time, one with bruising of grain and one with the strawing of strawberries at a market garden. East Lothian pig sheds produced high values for thermophilic actinomycetes and one East Lothian case was subsidiarily associated with a pig shed, the main hazard being released in a shed where grain bruising

Environment or Sample	Average Nos. actinomycetes per plate or per 10g. sample 46°C	60°C	Numbers and percentages of positive samples	Associated cases of farmer's lung	Cases of farmer's lung disease serologically positive to F.L.H.antigen
Ayr cattle courts	103.0		4/5(80)	2	0
Ayr dairy byres	42.0		39/48(81)	6	3
Ayr grain silos at harvest	-			3	1
Ayr grain processing sheds	-			1	1
Ayr market garden	-			1	0
E.L. pig sheds	64.0		4/5(80)	0 + 1	0
E.L. grain silos at harvest	-		-	3	0
E.L. grain processing sheds	-		-	1	0
Ayr bad hay	140.7	452.7	9/16(56)	5*	1
Ayr good hay	9.8	16.3	6/16(38)	3	2
Ayr barley straw	33.0	487.0	5/16(31)	1 + 2	
Ayr dusts	240.7	159.5	4/4(100)	1 + 7	
Ayr processed barley	18.6	49.7	2/10(20)		
Ayr barley(harvest)	-	-	-	3	2
E.L. dusts	44.2	20.4	3/5 (60)	1	
E.L. processed barley	17.0	4.0	3/10(30)		
E.L. barley harvest	-	-	-	3	0
Orkney hay	201.0	66.0	6/10(60)		
Orkney dusts	102.0	48.0	4/5 (80)		
Orkney hay or straw				20	18
Orkney grain				5 (6)	

TABLE 13: The types of farm environment and farm products associated with cases of farmer's lung disease in Ayrshire, East Lothian and Orkney and those containing the largest numbers of thermophilic actinomycetes expressed as:-

The average number per gravity Petri-dish or per 10g. sample

the number of positive plates or samples (numerator) per total number in group (denominator): also expressed as a percentage ( ).

+1 Subsidiary hazard

* figures after brackets indicate the number of cases involving multiple hazards. The number of cases, associated with each type of hazard or environment, serologically positive to farmer's lung hay (F.L.H.) antigen.

was taking place. The three remaining East Lothian cases were associated with bulk movement at harvest time.

ii) Farm products in Ayrshire and East Lothian.

Hay was claimed to be the major hazard relating to farmer's lung disease in eight out of the thirteen Ayrshire cases. In one case, grain processing, and in three both hay and grain processing were major hazards. In seven cases, the grain processing was a subsidiary hazard, i.e. was not thought by the worker to be the original cause of symptoms, but rendered the worker symptomatic on exposure. Barley straw was a main hazard in one case and subsidiary in two. Bulk barley movement at harvest time was the main hazard in three cases.

iii) Hays and dusts in Orkney

The main hazards implicated in the 31 Orkney cases of farmer's lung disease are shown in Table 13. Hay or straw was the main hazard in 20 cases, grain in 5 and both were implicated in 6 cases. Only a limited number of random samples of hay and dust was collected in Orkney, and these released large numbers of thermophilic actinomycetes. The results cannot be correlated with Orkney cases of farmer's lung disease, but the figures were comparable to those for equivalent Ayr samples (i.e. bad hays and dusts) and higher than those for E.L. samples

b) The relative occurrence of Micropolyspora faeni and Thermoactinomyces vulgaris.

Figure 16 demonstrates the efficiency of the apparatus described in Materials and Methods, 4, above (Figure 15) in isolating micro-organisms from samples of hay, straw etc., compared with direct plating. Both Petri-dishes contained nutrient agar plus cycloheximide (Appendix A) and were incubated at 46°C. Bacterial colonies completely covered the agar on the plate of the directly plated sample, and this probably inhibited growth of other organisms as only a few small colonies were otherwise evident on the



Figure 16: A comparison between two methods of estimating the microbiology of a sample -
a) by blowing a small quantity of air over the sample and collecting the airborne propagules on medium.
b) by plating a portion of the sample directly onto medium.
The medium consisted of ½ strength nutrient agar containing cycloheximide, and both plates were incubated at 46°C.

surface of the plate. On the other plate, numerous discrete colonies, predominantly of M. faeni from propagules blown off the sample by the simple method described above, are clearly seen. The colonies on the latter plate are probably more representative of the material which would be released into the air under normal working conditions.

The results given in Table 14 show that M. faeni singly or in combination with T. vulgaris occurred proportionately more frequently in Ayr than in E.L. samples. Hay, straw and dust were the richest sources of M. faeni in Ayrshire. T. vulgaris, however, occurred proportionately more frequently in E.L. than in Ayr samples and was obtained solely from bruised barley and dust. Only the hay samples from Orkney showed significant counts of M. faeni. The distribution of M. faeni correlates with the incidence of farmer's lung disease in all three counties (Table 7)

6. Serology

Three of the 6 cases of farmer's lung disease associated with dairy byres were serologically positive to standard farmer's lung hay (F.L.H.) antigen (Table 13). Of the remaining 7 cases, 2 were positive, 1 associated with a grain silo at harvest and the other with a grain bruising shed. Eight of the 13 Ayr cases and all E.L. cases were serologically negative. Of the 5 serologically positive cases, 1 was associated with hay, 2 were associated with both hay and grain and 2 with barley at harvest time. In Orkney, 13 of the 31 cases were serologically positive.

7. Statistics

A chi^2 test on all visibly good and bad hay samples from Ayr and E.L. with respect to thermophilic actinomycetes, showed that bad hays taken as a whole, gave a higher proportion of positive counts than good hays (Table 15). On a similar basis, Ayr hays, taken as a whole, or subdivided into 'good' or 'bad' gave more positive counts than equivalent E.L. hays. Total dusts gave more positive counts than total hays. All these values were greater than those which would be expected by chance. The number of positive counts from Ayr dusts and E.L.

Sample source, type and number	Thermophilic actinomycetes (G.A.)	Numbers positive for:-		
		M.faeni (N+C)	T.vulgaris (N+C)	M.faeni & T.vulgaris (N+C)
Ayrshire				
Dust (4)	4	3	2	2
Good hay (16)	6	3	2	2
Bad hay (16)	9	6	4	2
Barley straw (16)	5	4	4	3
Bruised barley (10)	2	1	1	1
TOTAL (62)	26(42%)	17(27%)	13(21%)	10(16%)
East Lothian				
Dust (5)	5	1	4	0
Good hay (10)	1	1	0	0
Bad hay (3)	1	1	0	0
Barley straw (10)	1	1	0	0
Bruised barley (10)	3	0	2	0
TOTAL (38)	11(29%)	4(11%)	6(16%)	0
Orkney				
Dust (5)	4	0	0	0
Hay (10)	6	3	0	0
TOTAL (15)	10(67%)	3(20%)	0	0

Table 14: The numbers and percentages of samples from Ayrshire, East Lothian and Orkney positive for Micropolyspora faeni, Thermoactinomyces vulgaris and total thermophilic actinomycetes.

G.A.: glycerol asparaginate agar

N+C: agar containing nutrient broth plus cycloheximide.

Samples compared	Samples with a higher proportion of + ve. counts for thermophilic actinomycetes.	Results of X^2 test.
Bad hays v good hays	Bad hays	$p<0.07$
Ayr hays v E.L. hays	Ayr hays	$p<0.01$
Ayr bad hays v E.L. bad hays	Ayr bad hays	$p<0.05$
Ayr good hays v E.L. good hays	Ayr good hays	$p<0.05$
Dusts v hays	Dusts	$p<0.01$
Ayr dusts v E.L. dusts	Ayr dusts	$p<0.2$

Table 15: Results of a X^2 test on the numbers of positive counts of thermophilic actinomycetes from Ayrshire and East Lothian hay and dust samples.

dusts showed little difference but the large difference in the mean values may be significent. If is impossible to say conclusively as the number of samples is very small.

Conclusions

1. The majority of the farming population in Ayrshire is employed on dairy farms and in East Lothian, on arable stock farms. (In Orkney, the majority of the farming population consisted of crofters cultivating small acreages of arable land.)

2. There is a positive correlation between the prevalence of farmer's lung disease (86,86 and 23 per 1000 in Orkney, Ayrshire and East Lothian) and the following:

i) The proportion of a small number of sputa from each region, from which colonies of thermophilic actinomycetes or of Aspergillus fumigatus were cultured.

ii) The proportion of equivalent sample groups of farm products from the three regions which contained thermophilic fungi and actinomycetes, notably M. faeni and T.vulgaris.

iii) The average number of thermophilic organisms per equivalent sample group.

iv) A less high standard of hay preservation in Orkney and of hay and grain in Ayrshire compared with that in East Lothian. (The thermophilic organisms from the small number of 'bad' hay samples in East Lothian were predominantly fungi but from the larger number of 'bad' samples from Ayrshire, were thermophilic actinomycetes).

v) The high values for the numbers of thermophilic organisms from farm environments in Ayrshire, compared with low values in East Lothian. This is examplified by a comparison between the very high values for Ayrshire dairy byres and the very low values for East Lothian cattle-courts.

3. The types of environments and products most frequently implicated in being associated with farmer's lung diease and which it was possible to survey, were those with the highest average numbers of thermophilic actinomycetes.

4. Hay and straw were the main hazards implicated in causing farmer's lung disease in Orkney and Ayrshire.

5. Grain was a substantial hazard in both Ayrshire and East Lothian and was the main hazard in all four East Lothian cases of the disease. Bruised barleys and dusts from bruising-machines were the only East Lothian samples with relatively high average values for thermophilic actinomycetes.

6. Environments such as green-houses and out-houses in both Ayrshire and East Lothian contained very low numbers of organisms, particularly of thermophilic organisms. Milking parlours, where foddering and bedding of animals did not take place also realised low counts.

7. There was no correlation between the distribution of species of mesophilic fungi in environments or farm products and association of these environments or products with farmer's lung disease.

8. Very high values for the numbers of colonies of mesophilic fungi from a wide range of samples from Ayrshire, East Lothian and Orkney and from environments in Ayrshire and East Lothian were recorded.

9. Thermophilic and thermotolerant fungi were most common in Ayrshire cattle-courts and byres but were rarely isolated elsewhere. Relatively high numbers of colonies of these organisms were isolated from bad hay samples and dusts from Ayrshire, East Lothian and Orkney. These organisms did not, therefore, demonstrate as close an association with farmer's lung disease as did thermophilic actinomycetes.

10. The simple method used to remove spores and other propagules from farm products appeared to be more accurate than direct plating in quantitatively estimating surface contaminants of samples. It had the disadvantage of not giving the total number of propagules present on the surface of the sample but provided a rapid, simple and economical way of estimating comparable fractions of the air-borne spore concentrations which a worker might breathe in whilst working with the material examined.

11. Forty per cent of the sera from diagnosed cases of farmer's lung disease were positive to standard F.L.H. antigen.

SECTION III

A STUDY OF THE MICROBIOLOGY OF SELECTED DAIRY FARMS IN AYRSHIRE WITH REFERENCE TO FARMER'S LUNG DISEASE.

A Study of the Microbiology of Selected Dairy Farms in Ayrshire with Reference to Farmer's Lung Disease.

The survey outlined in Section II of this thesis realised the high prevalence of farmer's lung disease in Ayrshire of 86 per 1000 of the farming population (Grant et al., 1972). The majority of the Ayrshire farming population (72%) and ten of the thirteen cases of farmer's lung disease found in the county were employed on dairy farms. For this reason, two groups of Ayrshire dairy farms were studied, one group with and one group without medically well-documented cases of farmer's lung disease. Names and addresses of known cases in the county, apart from those encountered on the survey, were provided by Dr. J.W.N. Duerden and Dr. L.A. Hardie, Irvine Central Hospital, Ayrshire.

Two farming activities which cause large concentrations of organic dust to be released and which were implicated in causing farmer's lung disease (Grant et al., 1972 : Section II) are the unbaling of hay and the bruising of grain. The study described below attempted to establish whether the microbiology of 'dusts' released in farmer's lung disease (F.L.D.) farms differed qualitatively or quantitatively from those in non-F.L.D. farms. Samples of the materials used by farmers in the course of their work were also collected for microbiological assessment.

Materials and Methods

1) Farms

In Ayrshire, six dairy farms each with a known case of farmer's lung disease (F.L.D.) and five dairy farms with no record of F.L.D. in the six years previous to 1972 were chosen for microbiological examination during March and April, 1972. One Perthshire dairy farm with a known case of F.L.D. was also studied. The twelve farms were of equivalent size (100-210 acres) and of similar agricultural practice.

2) Agricultural Methods on Farms Studied.

At each farm a questionnaire was filled in, describing the methods used for harvesting, storing and, in some cases, preserving hay and cereal crops.

3) Air sampling of farm environments.

The air of farm buildings was sampled using an 8-stage Andersen Sampler (Andersen, 1958 : Appendix B) run at an air-flow of 251./min. for periods of between 5 and 30 secs., depending on the apparent concentration of dust in the atmosphere to prevent either incomplete sampling of the air or overloading of the plates. The air in dairy byres of five F.L.D.farms (L.M.,S.B.,M.H.,K.M. and A.) and five non-F.L.D. farms (M.F.,M.T.,W.H.,B.M. and B.) in Ayrshire was sampled before and approximately 5 mins. after unbaling of hay and foddering of dairy cattle. The bruising sheds of four F.L.D. farms (S.B.,E.M.,K.M. and A.) and four non-F.L.D. farms (M.F.,W.H., B.M. and B) were air-sampled before and approximately 5 mins. after bruising or hammer-milling of barley had begun. The dairy byre and bruising shed of the farm in Perthshire (B.P.) were sampled before and after unbaling of hay and before and after the bruising of oats, respectively.

Procedure after unbaling of hay or bruising of grain.

Three sets of 8 disposable, polystyrene 9-cm., Petri-dishes containing media were placed consecutively in the Andersen Sampler (Appendix B) and removed immediately after air-sampling was completed (Appendix B). The three sets consisted of:

i) 2% malt agar (Appendix A), subsequently incubated at 26°C, in uncompartmented Petri-dishes; M

ii) Petri-dishes divided into three compartments (Appendix B) containing:

a) glycerol asparaginate agar (Appendix A); G.A.

b) 2% agar containing half-strength nutrient broth (Oxoid) and cycloheximide at a concentration of 500 mg/l., (Appendix A); N + C.

c) 2% tap water agar; T

subsequently incubated at 46°C.

iii) as ii) but incubated at 60°C.

Procedure before unbaling of hay or bruising of grain

Four M plates, subsequently incubated at 26°C were placed in positions 0 to 3 in the Andersen Sampler (Appendix B) and four 3-compartmented plates containing G.A., N + C and T media, subsequently incubated at 46°C were placed in positions 4 to 7 to give an estimation of the microbiology of the air at apparently low concentrations of propagules.

The gravity Petri-dish technique was exploited by exposing two 9-cm Petri-dishes containing M and N + C agar media, subsequently incubated at 26°C and 46°C, for two minutes while air-sampling by the Andersen Sampler was taking place.

Counting and identification of fungal and actinomycete colonies.

Total counts of colonies on all plates were made after 48 hours according to the positive hole conversion and microscope methods of Andersen, 1958 (Appendix B). Mesophilic fungi on malt plates were identified after 7 to 10 days (Appendix D). Thermophilic fungi, on G.A. plates were identified after 4 to 5 days (Appendix D) but if growth was insufficient for identification, they were subcultured and grown for 10-14 days.

Actinomycetes and bacteria grown at 26°C were not further idenitified but thermophilic actinomycetes were initially grouped according to the colony characteristics of colour, shape, size and type of sporulation after 4-5 days incubation at 46°C and 60°C. The more common organisms

were identified to generic level, used as a source of antigens and examined by electron microscopy. Thermoactinomyces vulgaris was identified by colony characteristics (Figure 17) and by growth on selective medium containing cycloheximide and novobiocin (Appendix A)., (Cross, 1968). Colonies of Micropolyspora faeni (Figure 18) were identified by comparison with type cultures received from Dr. J. Lacey, Rothamsted Experimental Station, Harpenden, Herts. and from Dr. Christine Dawson, University of Glasgow Veterinary School, and also from the description of Cross et al., 1968.

The total number of colonies isolated at each temperature and the numbers of colonies of different species or groups of mesophilic and thermophilic fungi and of thermophilic actinomycetes were recorded. From these results, the numbers of different types of culturable propagules isolated per cubic metre of air was calculated.

4. Sampling of hay, grain and dust from farm environments.

Samples of hay, whole grain, bruised or hammer-milled grain and dust off bruising and milling machines were collected at the time of unbaling, bruising or milling. They were placed in weighed, sterile 41, tin cans and the weight of each sample was determined.

A length of stiff polythene tubing was attached to a fitment made on the lid of one of the tins. The other end of the tube was attached to the orifice at the top of the Andersen sampler. By means of the modified lid, each sample was connected, in turn, to the Andersen sampler and was shaken vigorously on a Griffin Flask Shaker for 30 secs. A polythene bag was placed round the tin and made airtight. Air was pumped out of the tin, over the plates in the sampler, until all the air containing propagules released from the sample was removed and the tin buckled. The colonies of sets of malt plates incubated at 26°C and 3-compartmented plates (as in 3 above), incubated at 46°C were counted after 48h. and idenitified as in 3 above. The numbers of

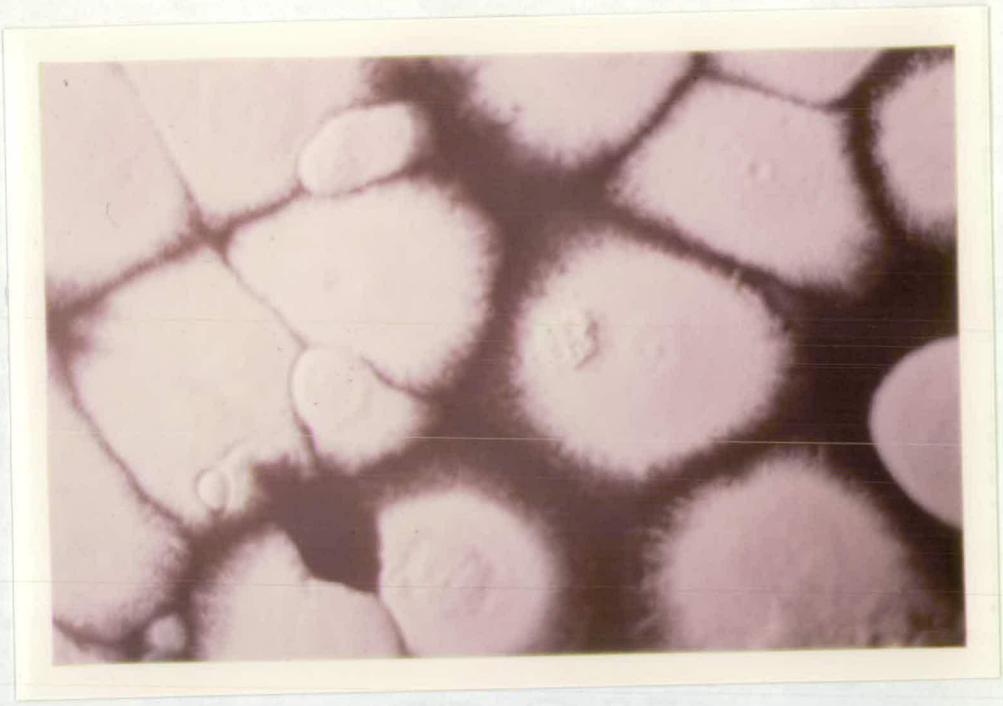


Figure 17: Colonies of Thermoactinomyces vulgaris grown at 46°C on ½ strength nutrient agar containing cycloheximide.

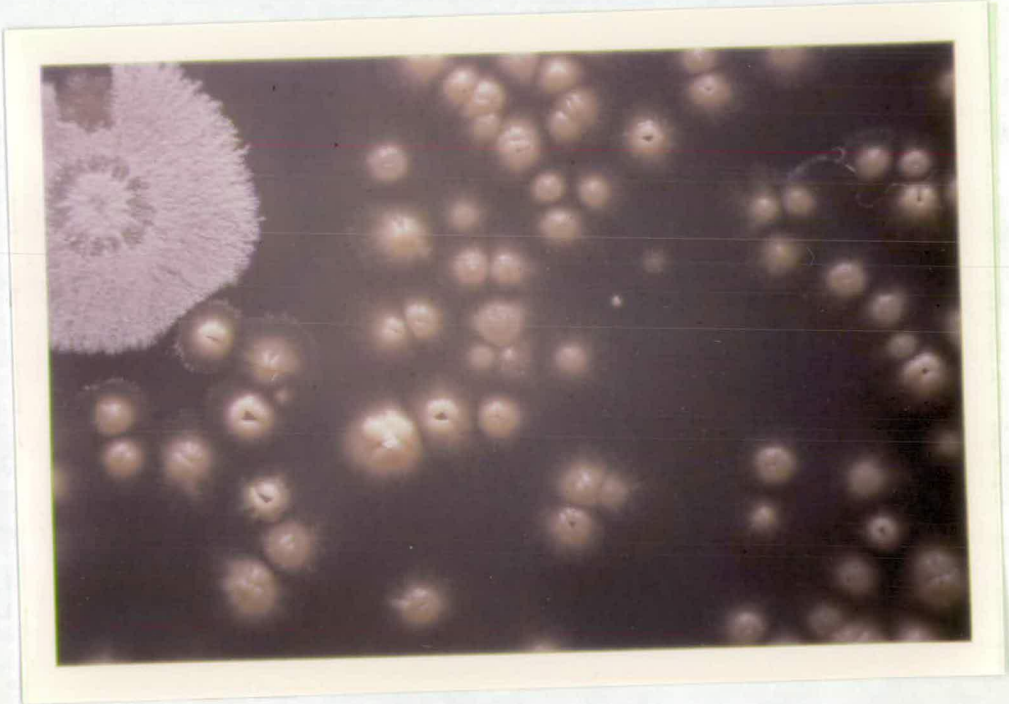


Figure 18: Colonies of Micropolyspora faeni (yellow colonies) grown at 46°C on ½ strength nutrient agar containing cycloheximide.

different types of culturable propagules released per standard weight of each sample type (e.g. per 10g. of hay) was calculated.

Results

1. Method of harvesting and storing hay and cereal crops

a) Hay

On all farms, hay was baled from the swath and on two farms (K.M. and M.F.) was artificially dried. On one farm (B.P.) half the hay crop was artificially dried and stored indoors while the other half was stacked in the open, directly from the field. Only one other farm (M.F.) had half of the hay crop stacked in the open. All other hay was stored indoors.

b) Cereal Crops

All farms except M.H. and M.T. grew their own barley crop. All barley was combine-harvested and the straw was baled and used for bedding animals. On two farms, both non-FLD, oats were binder cut, stored in the sheaf and used for fodder.

The various methods of storage of barley grain (and in one case, oats) are shown in Table 16.

2. The concentration of viable propagules in the air of dairy byres before and after unbaling of hay and in bruising sheds before and after bruising of grain.

a) Mesophilic fungi and actinomycetes on malt agar plates.

After unbaling of hay and bruising of grain, the number of mesophilic organisms isolated on plates 0 to 3 of the Andersen Sampler gave higher values per cubic metre of air than those recorded before these activities took place (Table 17). There was, however, considerable variation in the differences between the two counts recorded, from building to building. The largest proportions of colonies of mesophilic organisms were isolated on plates 0 to 3 of the Andersen Sampler after both unbaling of hay and bruising of grain with only three exceptions (Table 17). At farm A., only 17% of the total number of colonies isolated was on plates 0 to 3 after unbaling of hay. However, the

		METHODS OF STORAGE					
	FARM	1	2	3	4	5	6
FLD	L.M.	+ P				+	+ P
	S.B.			+ P			
	M.H.						+
	K.M.	+			+ P		
	A						+
	B.P.		+ O		+		
	E.M.					+	
NON-FLD	M.F.		+				
	M.T.						
	W.H.	+					
	B.M.					+	
	B.	+				+	

Table 16: Methods of storage of barley grain and oats (O) on 7 farmer's lung disease (FLD) farms and 5 non-FLD farms.

1. Dry, from field, in bulk
2. After artificial drying, in bulk
3. Moist in bulk
4. Moist in air-sealed silo
5. Moist, in air-sealed polythene bags
6. Dry, from field, in jute bags.

P. Propionic acid added as preservative.

		NO. OF COLONIES ISOLATED PER CUBIC METRE BY THE ANDERSEN SAMPLER (A.S.) X 10 -3				G.P.D.	A.S.	G.P.D.
		PLATES 0 to 3	PLATES 0 to 3(A)	PLATES 0 to 7(B)	A/B(%)	No.COLS PER PLATE	TOTAL NO. OF FUNGAL SPECIES OR GROUPS	TOTAL NO. OF FUNGAL SPECIES OR GROUPS
	FARM	2b	2c	2c	2c	2c	2c	2c
FLD	L.M.	33	483	866	56	92	11	4
	S.B.	4	10	16	63	43	8	8
	M.H.	15	2,682	4,810	56	6,949	13	12
	K.M.	N.R.	847	940	90	229	13	8
	A.	47	348	2,003	17	427	9	6
	B.P.	16	2,700	5,418	50	889	14	6
NON-FLD	M.F.	65	809	1,340	60	1,090	11	7
	M.T.	17	1,012	1,360	74	1,505	9	6
	W.H.	N.R.	1,160	2,099	55	1,029	14	8
	B.M.	18	1,872	2,169	86	4,092	14	10
	B.	16	1,224	2,607	47	2,136	15	10
		1a	1b	1b	1b	1b	1b	1b
FLD	S.B.	8	219	256	86	347	9	7
	E.M.	4	7,411	8,348	89	1,840	6	2
	K.M.	7	9,162	12,284	75	8,064	5	2
	A.	95	207	321	64	63	9	8
	B.P.	10	28	70	37	134	14	9
NON-FLD	M.F.	64	63,686	88,861	72	2,560	5	2
	W.H.	56	5,180	8,676	60	2,763	2	3
	B.M.	30	535	589	91	929	13	10
	B.	5	9,292	14,728	63	6,680	5	3

Table 17: The number of colonies and of species of mesophilic fungi and actinomycetes isolated on malt agar plates by the Andersen Sampler and gravity Petri-dish (G.P.D.) technique from the air of dairy byres before (2b) and after (2c) unbaling of hay and from bruising sheds before (1a) and after (1b)bruising of grain.

Plates 0 to 3: The number of colonies isolated per cubic metre of air on the upper four plates of the 8-stage Andersen Sampler.

Plates 0 to 7: The total number of colonies isolated per cubic metre of air by the 8-stage Andersen Sampler.

A/B(%): The percentage of the total number of colonies isolated by the Andersen Sampler on pates 0 - 3.

majority of mesophilic colonies from this environment were actinomycetes. On two other farms the numbers of colonies on plates 0 to 3 were 37% and 47% of the total. From the other seventeen environments, out of a total of twenty, 50% to 91% of the total colonies isolated were collected on plates 0 to 3, indicating that the use of these plates in estimating the original concentration of mesophilic organisms was valid.

b) Thermophilic actinomycetes and bacteria isolated on plates of nutrient agar containing cycloheximide.

With four exceptions, the concentrations of thermophilic actinomycetes and bacteria in dairy byres and bruising-sheds were higher after unbaling of hay and bruising of grain, respectively than before these activities had begun (Table 18). As was the case for the numbers of mesophilic organisms in (a) above, there was considerable variation in the differences between the two counts, from building to building. The largest proportions of colonies of thermophilic actinomycetes and bacteria isolated after unbaling of hay or bruising of grain (55% to 91%) were recovered on plates 4 to 7 of the Andersen sampler in thirteen of the twenty environments sampled. From two environents, 40% to 50% of the total number of colonies isolated was on plates 4 to 7, from three environments, 30% to 40% and from two others 10% to 15%; the seven environments all yielded low total values. The results indicate, however, that it was valid to use plates 4 to 7 to give an indication of the numbers of thermophilic actinomycetes and bacteria present in the air sampled.

The numbers of colonies isolated per cubic metre of air by the Andersen Sampler, compared with the numbers of colonies isolated by the gravity Petri-dish (G.P.D.) technique.

Values obtained by the gravity Petri-dish technique on exposure of plates for 2 minutes are, in general, relatively high when results

		ANDERSEN SAMPLER (A.S.)				G.P.D.	A.S.	G.P.D.
	FARM	PLATES 4 to 7 2b	PLATES 4 to 7(A) 2c	PLATES 0 to 7(B) 2c	A/B(%) 2c	NO. COLS PER PLATE 2c	TOTAL NO. OF COLONY TYPES 2c	TOTAL NO. OF COLONY TYPES 2c
FLD	L.M.	49	725	796	91	N.R.	10	N.R.
	S.B.	8	12	18	67	59	12	7
	M.H.	62	179	190	94	451	13	3
	K.M.	N.R.	13	23	57	60	9	4
	A	70	350	633	55	N.R.	16	N.R.
	B.P.	2	471	564	84	411	13	6
NON-FLD	M.F.	0	1	10	10	46	8	6
	M.T.	1	348	402	87	400	11	4
	W.H.	N.R.	25	58	43	120	11	3
	B.M.	25	49	88	56	249	12	4
	B.	16	2	14	14	113	6	5
		1a	1b	1b	1b	1b	1b	1b
FLD	S.B.	582	1,607	1,664	97	180	12	N.R.
	E.M.	5	9	12	75	62	8	6
	K.M.	1	1,556	1,811	86	1,712	8	1
	A.	46	5	16	31	9	7	2
	B.P.	60	15	46	33	99	10	4
NON-FLD	M.F.	1	85	141	60	15	11	3
	W.H.	1	41	93	44	62	8	6
	B.M.	13	1	3	33	19	6	5
	B.	22	15	21	71	22	4	3

Table 18: The number of colonies and of colony types of thermophilic actinomycetes and bacteria isolated on N + C agar plates by the Andersen Sampler and gravity Petri-dish (G.P.D.) technique from the air of dairy byres before (2b) and after (2c) unbaling of hay and from bruising sheds before (1a) and after (1b) bruising of grain.

Plates 4 - 7: The number of colonies isolated per cubic metre of air on the lower four plates of the 8-stage Andersen Sampler.

Plates 0 - 7: The total number of colonies isolated per cubic metre of air by the 8-stage Andersen Sampler

A/B(%): The percentage of the total number of colonies isolated by the Andersen Sampler on plates 4 - 7.

obtained by the Andersen Sampler, run for 5 to 15 secs. at equivalent times, in the same environment are high (Tables 17 and 18). G.P.D. results are also relatively low when Andersen Sampler results are low although there is no arithmetic relationship between the numbers of colonies obtained by the two methods.

The numbers of species or groups of organisms isolated by the Andersen Sampler compared with those obtained by the G.P.D. technique.

Gravity Petri-dishes containing malt agar, exposed for 2 minutes isolated fewer species or groups of mesophilic organisms than plates from the Andersen Sampler, run at an air-flow of 25 1/min for between 5 and 15 secs., in the same environment (Table 17). (Mesophilic actinomycetes were counted as one group). This situation also held for the numbers of colony types of thermophilic actinomycetes and bacteria isolated on N.+.C. plates (Table 18). The total numbers of species or groups of mesophilic organisms isolated on malt plates by the Anderesen Samples and G.P.D. technique were added together and it was established that the G.P.D's isolated 66% of the number of species or groups recovered by the Andersen Sampler. On the same basis, for equivalent environments (omitting those for which no G.P.D. results were obtained), the G.P.D. technique isolated 45% of the colony types of thermophilic bacteria and actinomycetes recovered by the Andersen Sampler.

3. A comparison between the numbers of thermophilic organisms isolated from farm buildings on plates of nutrient agar containing cycloheximide (N.+.C) and glycerol asparaginate agar (G.A) incubated at 46°C and those isolated after incubation of equivalent plates at 60°C.

The numbers of thermophilic actinomycetes and bacteria isolated from the same sample of air byboth N.+.C. and G.A. agar plates incubated at 46°C and the numbers isolated from plates incubated at 60°C are shown in Table 19. The numbers of thermophilic fungi isolated on G.A. incubated

	FARM	N+C 46°C	G.A.-F 46°C	N+C 60°C	G.A.-F 60°C	G.A. 46°CF	G.A. 60°CF
2c FLD	L.M.	796	N.R.	1,311	N.R.	N.R.	N.R.
	S.B.	18	4	165	4	4	6
	M.H.	190	36	1,670	37	14	9
	K.M.	23	16	34	13	0	0
	A.	633	476	715	319	457	213
	B.P.	564	41	255	71	40	184
NON-FLD	M.F.	10	14	1,299	27	40	21
	M.T.	402	88	686	25	79	14
	W.H.	58	37	39	19	26	0
	B.M.	88	5	29	7	0	1
	B.	14	13	55	0	6	1
1b FLD	S.B.	1,664	62	687	68	30	2
	E.M.	12	22	57	7	14	1
	K.M.	1,811	289	2,244	17	58	0
	A.	16	9	59	0	3	3
	B.P.	46	1	42	0	15	5
NON-FLD	M.F.	141	50	15	20	2	2
	W.H.	93	11	39	5	8	4
	B.M.	3	3	8	8	0	0
	B.	21	1	356	2	0	2

Table 19: The total number of colonies X 10^{-3} of actinomycetes and bacteria isolated /m^3 by the Andersen Sampler on nutrient agar containing cycloheximide (N+C) and on glycerol asparaginate agar (G.A.-F), incubated at 46°C and 60°C. The number of colonies of fungi (F) isolated on glycerol asparaginate agar (G.A.) incubated at 46°C and 60°C.

FLD: farmer's lung disease

NON-FLD: non 'farmer's lung disease'

2c : in dairy byres after unbaling of hay

1b : in bruising sheds after bruising of grain.

at 46°C and 60°C after unbaling of hay and bruising of grain in farm buildings are also included. The sample of air passed over the plates of the Andersen Sampler which were incubated at 46°C was taken approximately 45 seconds before that relating to the set incubated at 60°C.

i) Thermophilic actinomycetes and bacteria isolated on plates of N.+.C. agar.

After unbaling of hay, farms S.B. and K.M. had the lowest values for FLD farms at both 46°C and 60°C. Farm L.M. realised a larger total number of colonies and a larger number of colonies of M.faeni per cubic metre of air (Table 20) at 60°C than at 46°C. The greatest difference between the numbers of colonies isolated at the two temperatures for an FLD farm after unbaling of hay was at farm M.H. with 190 colonies/m^3 at 46°C and 1,670 colonies/m^3 at 60°C of which 1,453 were colonies of M.faeni. All other farms, after unbaling of hay, gave results of the same order for plates incubated at 46°C and 60°C except non-FLD farm, M.F., with 1,299 colonies isolated after incubation at 60°C, of which 1,052 were of colony type 801 and 52 were colonies of M.faeni, indicating a very different microbiology from that of the plates incubated at 46°C. At farm M.T. there were slightly more colonies of M.faeni isolated from plates incubated at 60°C (55/m^3) than from those incubated at 46°C (12/m^3).

After bruising of grain, the FLD farms S.B. and K.M., with the lowest values of thermophilic organisms isolated on N.+.C. plates incubated at both 46°C and 60°C after unbaling of hay, gave very high counts at 60°C and at 46°C. The highest concentration of thermophilic actinomycetes and bacteria (2,244/m^3) isolated from K.M. plates incubated at 60°C coincided with the highest concentration (2,020/m^3) of propagules of M.faeni (Table 21). A slightly higher

TEMPER-ATURE	TYPE OF PROPAGULE	FLD FARMS						NON-FLD FARMS				
		L.M.	S.B.	M.H.	K.M.	A	B.P.	M.F.	M.T.	W.H.	B.M.	B
46°C	TOTAL	796	18	190	23	633	564	10	402	58	88	14
	M. FAENI	308	2	51	6	45		3	12	4	2	1
	T. VULGARIS		2	10		57	11	2	4			
	801											
	802/5				+	38			16	2		
	401	146	2	19		6	284					
	WHITE ACTINOMYCETES	103	+	49	5	310	40	1	358	18	11	1
	BROWN ACTINOMYCETES	183	8	17	+	57	45	1	4	7	4	
	OTHER ACTINOMYCETES			13	1	6	46		4	11	11	+
	501/2 BACTERIA	16	2	30	4	13	132	1	4	15	60	10
	OTHER BACTERIA	40	+		7	100	6	1		1		1
60°C	TOTAL	1,311	165	1,670	34	715	255	1,299	686	39	29	55
	M. FAENI	1,193	N.R.	1,453	24	11		52	55	2		
	T. VULGARIS	3			+	36	110	39	20	2	8	16
	801							1,052		3		
	802/5				1	72			7			
	BLUE/GREY/GREEN ACTINOMYCETES	+		9		1	2					
	WHITE ACTINOMYCETES	24		33	1	236	10	130	569	19		3
	BROWN ACTINOMYCETES			67	2	215	23		7	4	1	
	OTHER ACTINOMYCETES	52		24	+	7	2	13	7			4
	501/2 BACTERIA	13		84	+	1	99	13	14	9		
	OTHER BACTERIA	26			4	136	9		7		20	32

Table 20: The total numbers of viable propagules X 10^{-3}, per cubic metre of air, and the numbers of different types of thermophilic actinomycetes and bacteria cultured on N+C agar incubated at 46°C and 60°C after unbaling of hay in dairy byres.

FLD: 'farmer's lung disease'
NON-FLD: non - 'farmer's lung disease'
N+C: nutrient agar containing cycloheximide
+: less than 1 propagule/m^3.

801: M. faeni variant?
802/5: M. faeni variant
401: BLUE/GREY/GREEN ACTINOMYCETES
+ : less than 1 viable propagule per m^3

TEMPER-ATURE	TYPE OF PROPAGULE	FLD FARMS					NON-FLD FARMS			
		S.B.	E.M.	K.M.	A	B.P.	M.F.	W.H.	B.M.	B
46°C	TOTAL	1,664	12	1,811	16	46	141	193	3	21
	M. FAENI	90	1	427	2	3	32	13	+	-
	T. VULGARIS	-	-	-	-	2	7	1	-	-
	801	6	-	-	-	-	2	1	-	-
	802/5	-	-	-	2	-	70	-	-	-
	401	4	1	-	-	1	-	-	-	-
	WHITE ACTINOMYCETES	316	2	1,268	2	6	-	140	-	6
	BROWN ACTINOMYCETES	133	-	36	-	2	11	5	+	-
	OTHER ACTINOMYCETES	-	1	-	2	1	7	-	-	2
	501/2 BACTERIA	1,098	5	80	7	31	5	33	2	13
	OTHER BACTERIA	17	2	-	1	-	7	-	+	-
60°C	TOTAL	687	57	2,244	59	42	15	39	8	356
	M. FAENI	192	37	2,020	2	-	1	10	-	-
	T. VULGARIS	14	13	76	-	-	-	2	-	-
	801	-	-	-	-	-	-	-	-	-
	802/5	7	-	-	1	-	-	-	-	3
	BLUE/GREY/GREEN ACTINOMYCETES	-	-	-	-	-	-	-	-	-
	WHITE ACTINOMYCETES	254	-	76	2	6	1	4	-	39
	BROWN ACTINOMYCETES	76	-	-	-	2	1	12	-	3
	OTHER ACTINOMYCETES	62	1	-	6	27	4	-	-	15
	501/2 BACTERIA	-	1	90	-	7	1	11	-	43
	OTHER BACTERIA	82	5	2	48	-	7	-	8	253

Table 21: The total numbers of viable propagules X 10^{-3}, per cubic metre of air, and the numbers of different types of thermophilic actinomycetes and bacteria cultured on N+C agar incubated at 46°C and 60°C after bruising of grain in bruising sheds.

FLD: 'farmer's lung disease'
NON-FLD: non - 'farmer's lung disease'
N+C: nutrient agar containing cycloheximide
+: less than 1 propagule/m3
801: M. faeni variant?
802/5: M. faeni variant
401: BLUE/GREY/GREEN ACTINOMYCETES
+: less than 1 viable propagule per m^3

total number of organisms was recovered from farms E.M. and A. at 60°C compared with that at 46°C. From the former farm, this increase consisted mainly of colonies of M.faeni and from the latter, of colonies of bacteria. Farm B.P. realised similar counts at the two temperatures but the types of colony making up the total were different. The non-FLD farms M.F. and W.H. released fewer colonies onto the plates incubated at 60°C than onto those incubated at 46°C. Colonies of M.faeni and 802/5 were not recorded from the plates incubated at 60°C from M.F. although 102 colonies /m^3 had been recovered on plates incubated at 46°C. The difference between counts for W.H. from plates incubated at 46°C and 60°C were largely due to the small numbers of colonies of white actinomycetes isolated at 60°C (Table 21). At farm B, this situation was reversed and large numbers of colonies of bacteria isolated at 60°C caused a larger number of colonies to be isolated at that temperature (Table 21).

ii) The number of colonies of thermophilic actinomycetes and bacteria isolated on plates of N.+.C. agar compared with the numbers isolated on plates of G.A. agar incubated at both 46°C and 60°C.

Results for the numbers of colonies of actinomycetes and bacteria recovered on N.+.C. and G.A. agar for the same sample of air (Table 19), demonstrate that the numbers isolated on G.A. agar are generally lower and in some cases, considerably lower, than those obtained on N.+.C. agar. The largest difference, in this respect, was recorded from farm K.M., after bruising of grain, with values of 2,244 colonies /m^3 on N.+.C agar and 17 colonies/m^3 on G.A. agar from the same plates incubated at 60°C. Differences between the less high values obtained on N + C and those on G.A.

were less marked.

iii) The numbers of thermophilic fungi isolated on plates of G.A. agar incubated at 46°C compared with the numbers isolated on those incubated at 60°C.

In general, the numbers of thermophilic fungi isolated at 60°C were approximately 50% or less of the numbers isolated at 46°C for the same environment (Table 19). In only one farm building, the dairy byre of B.P., after unbaling of hay, was there a significantly higher value recorded after incubation at 60°C than that at 46°C. The highest counts recorded for both temperatures were from farm A, after unbaling of hay (457/m^3 at 46°C, 213/m^3 at 60°C).

4. Mesophilic and thermophilic organisms isolated from farm buildings and from hay, grain and dust samples.

In the following account of the numbers of different species or groups of mesophilic fungi isolated from both farm environments and samples of hay, grain and dust mesophilic Aspergilli were divided into the collective groups described in Raper and Fennel, 1965 (Appendix D). Colonies described as being of A.glaucus for example, were therefore members of the group A.glaucus.

Mesophilic fungal spcies and groups isolated from farm buildings and from samples of hay, grain and dust were:

Acremoniella atra, Alternaria spp., Aspergillus flavus, A.fumigatus, A. glaucus, A. nidulans, A.niger, A. ochraceus, A.ornatus, A.restrictus. A. terreus, A. ustus, A. versicolor, Aureobasidium spp., Botryotrichum spp., Botrytis cinerea, Chaetomium spp., Chrysosporium spp., Cladosporium spp., Doratomyces sp., Epicoccum spp., Fusarium spp., Geotrichum spp. Mucorales, Mycelia sterilia, Paecilomyces spp., Penicillium spp., Scopulariopsis spp., Sporothrix spp., Trichoderma viride, Trichothecium roseum, Verticilluim spp. and Yeasts.

Identified thermophilic fungal species and groups were:

Aspergillus fumigatus, Humicola spp. and Mucorales.

As seen in Tables 23, 24 etc., thermophilic actinomycetes were classified according to colony characteristics (Appendix D). Colony types 802/5 were taken to be strains of M. faeni and colonies of the 801 types were very small, unidentified colonies of actinomycetes which may have been M. faeni variants. Colony types 401 were blue/grey/green and were probably members of the species Saccharomonospora viridis (Nonomura and Ohara, 1971), formerly Thermopolyspora glauca Henssen or of the species Thermoactinomyces glaucus Henssen. Both species are discussed by Cross and Goodfellow, 1973.

Bacterial colony types 501/2 were cream to pink in colour and large and spreading. Other, less common, bacterial types were classified together and had, in general, smaller colonies with entire edges.

Photographs showing colonies of mesophilic fungi grown on malt agar plates and colonies of thermophilic actinomycetes, bacteria and fungi grown on compartmented plates containing N + C and G.A. agars, after incubation at 46°C, are seen in Figures 19 and 20. The plates were from position 5 of the Andersen Sampler and the colonies were from samples of whole and bruised barley and dust off the bruising-machine from FLD farm, S.B. The mesophilic fungi were predominantly colonies of Penicillium spp., demonstrating the large numbers of propagules of these species released from whole and bruised barley. The very small colonies on the N + C portion of the plates incubated at 46°C were predominantly of M. faeni. Colonies of white and brown actinomycetes can be seen as well as those of bacteria, notably the cream/pink coloured 501/2 type. Mature colonies of thermophilic fungi can be seen on the G.A. sections of the plates from whole and bruised barley, obscuring colonies of actinomycetes and bacteria.

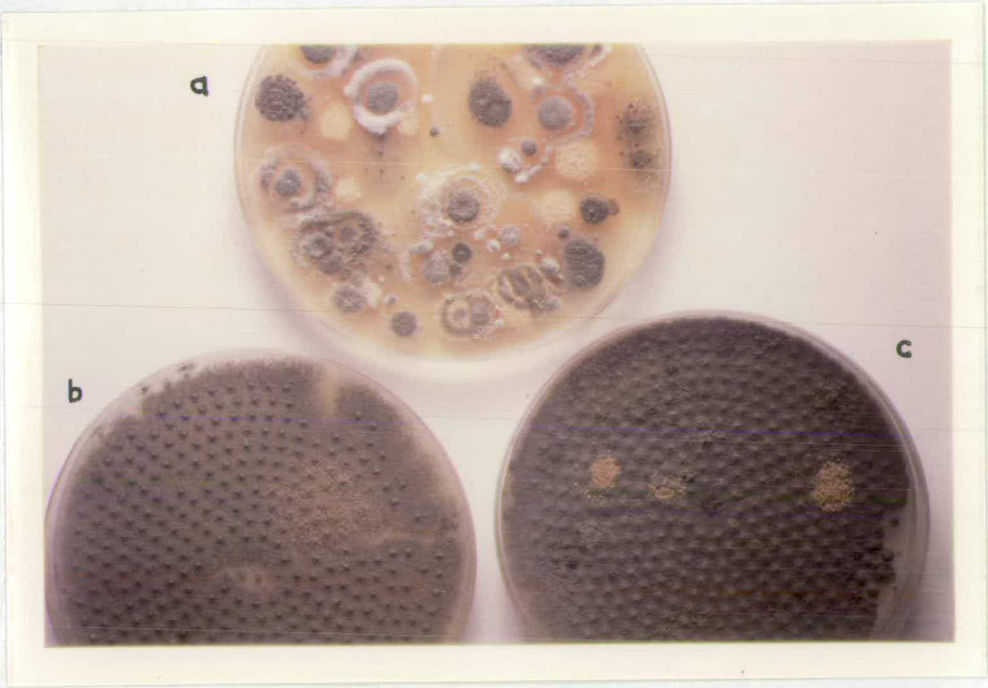


Figure 19: Colonies of airborne organisms isolated on 2% malt agar, incubated at 26°C from samples of a)whole barley, b)bruised barley and c)dust from a bruising machine by using an Andersen sampler. The Petri dishes were all from position 5 of the sampler(8-stage).

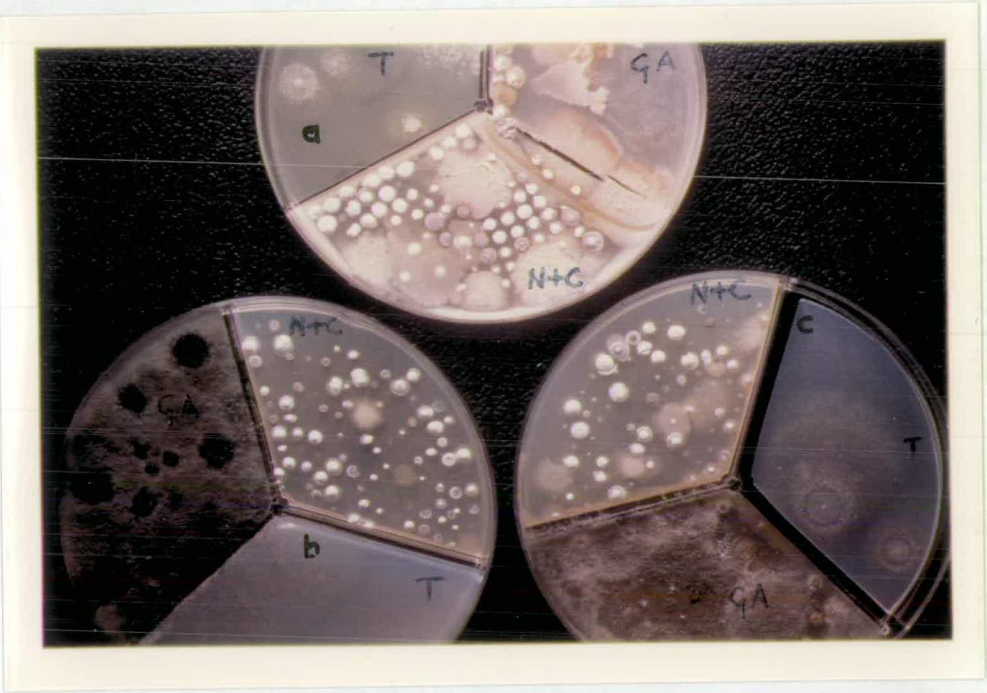


Figure 20: Colonies of airborne organisms isolated on agar media incubated at 46°C from samples of a) whole barley, b) bruised barley and c) dust from a bruising machine by using an Andersen sampler. The Petri dishes were all from stage 5 of the 8-stage sampler.

G.A. = glycerol-asparaginate agar

N+C = ½ strength nutrient agar containing cycloheximide

T = tap water agar

A. Organisms isolated from the air of farm buildings by the Andersen Sampler.

a) Mesophilic organisms isolated on plates of malt agar.

The numbers of colonies of mesophilic organisms isolated per cubic metre of air on malt agar plates by the Andersen Sampler after unbaling of hay in dairy byres and after bruising of grain in grain-bruising sheds are shown in Graph 20. The numbers of colonies of different groups or species of mesophilic fungi and of mesophilic actinomycetes are recorded in Tables 22 and 23.

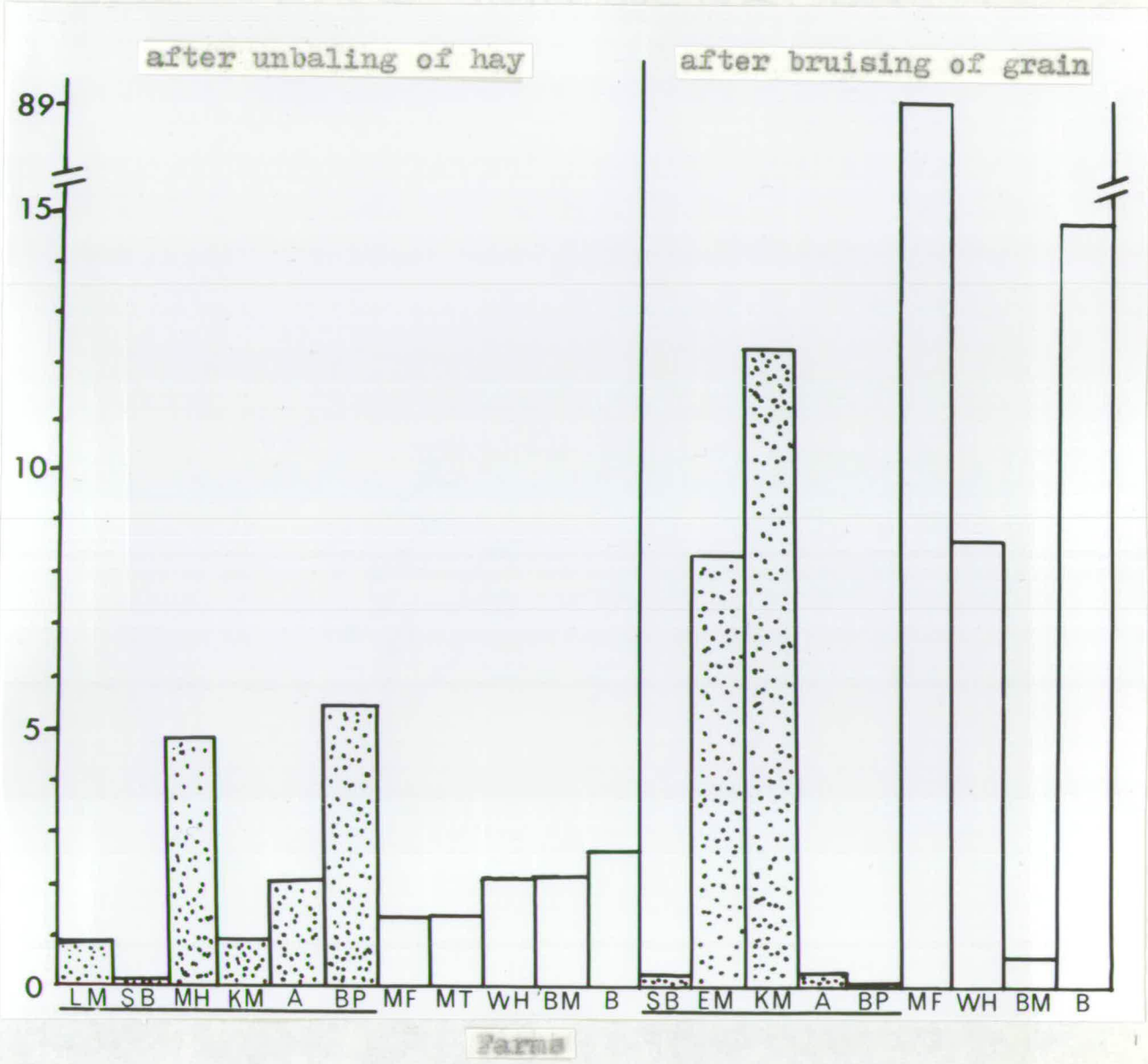
i) Mesophilic organisms isolated from dairy byres.

There is considerable variation in the numbers of colonies isolated from dairy byres on FLD farms but the numbers from all non-FLD dairy byres are of approximately the same magnitude, intermediate to the values obtained from the air of FLD dairy byres (Graph 20 and Table 22). The air from dairy byres of two FLD farms, M.H. and B.P., realised the highest values of 4,810 x $10^3/m^3$ and 5,418 x $10^3/m^3$, respectively.

Fungi, predominantly of Aspergillus spp. were the most common mesophilic organisms (Table 22) but the colonies isolated from one FLD farm, A., were almost all of actinomycetes. A.glaucus was found in appreciable numbers in all but four environments (those of the FLD farms, L.M., S.B., A and B.P.) and predominated in the air of three non-FLD dairy byres (M.F., B.M. and B). A glaucus and A versicolor predominated in M.H., A.terreus in L.M. and M.T. and A. fumigatus, along with yeasts and members of the Mucorales were the most common fungi isolated from the B.P. dairy byre. From only one dairy byre, on the non-FLD farm, W.H., were colonies of Penicillium spp. the most common.

ii) Mesophilic organisms isolated from bruising sheds.

There was no significant differentation between the numbers of mesophilic colonies isolated from FLD and non-FLD bruising-sheds



Graph 20: The number of colonies isolated on 2% malt agar per cubic metre of air from dairy byres after unbaling of hay and from bruising sheds after bruising of grain by using the Andersen sampler on farmer's lung disease (FLD) and non-FLD farms

Number of colonies isolated at 26°C per cubic metre of air X 10^{-6}

▨: FLD farm
□: non-FLD farm

TYPE OF PROPAGULE	FLD FARMS						NON-FLD FARMS				
	L.M.	S.B.	M.H.	K.M.	A.	B.P.	M.F.	M.T.	W.H.	B.M.	B.
TOTAL	866	16	4,810	940	2,003	5,418	1,340	1,360	2,099	2,169	2,607
ASPERGILLI (GROUPS)											
A.FUMIGATUS	9	+	+	-	40	1,517	3	14	273	1	-
A.GLAUCUS	165	9	1,732	808	3	18	1,059	585	336	2,017	1,512
A.NIDULANS	26	-	+	-	40	813	-	+	2	-	-
A.RESTRICTUS	-	-	673	-	-	-	255	9	9	108	652
A.TERREUS	260	+	+	+	17	18	-	694	9	4	-
A. VERSICOLOR	43	+	1,780	94	-	54	-	2	-	4	339
AUREOBASIDIUM SP	-	-	+	+	40	-	-	+	21	2	6
GEOTRICHUM SP	-	-	-	-	-	18	-	-	8	+	+
MUCORALES	34	-	-	+	40	1,029	+	14	-	3	-
PENICILLIUM SP	17	+	481	9	80	488	7	41	1,175	13	26
YEASTS	208	+	144	4	20	1,409	7	-	63	10	15
OTHERS	+	2	-	-	-	-	-	-	-	-	-
ACTINOMYCETES	104	4	+	9	1,723	54	5	-	189	5	2

Table 22: The numbers of viable propagules X 10^{-3} of different species of mesophilic fungi and of mesophilic actinomycetes cultured on malt agar, incubated at 26°C. Results are expressed per cubic metre of air and are from dairy byres after unbaling of hay.

FLD: 'farmers' lung disease'
NON-FLD: non- 'farmer's lung disease'
+: less than 1 viable propagule per m^3

(Graph 20). Three F.L.D. farms (S.B., A and B.P.) and one non-F.L.D. farm (B.M.) yielded comparatively few colonies while two farms from each category (F.M./K.M. and W.N./B.) yielded between 8,000 x $10^3/m^3$ and 15,000 x $10^3/m^3$ mesophilic organisms, values higher than any obtained after unbaling of hay. An even greater figure of 88,861 x 10^3 colonies/m^3 (Graph 20 and Table 23) was recorded for a non-F.L.D. bruising shed, M.F., and consisted predominantly of colonies of yeasts and a Geotrichum sp. The air of this bruising-shed also contained significant numbers of propagules of A. candidus (548 x $10^3/m^3$). On one other farm, B., yeasts were predominant but colonies of Penicillium spp. made up the largest proportion of all those isolated after grain bruising.

b) Thermophilic actinomycetes and bacteria isolated on plates of nutrient agar containing cycloheximide (N + C) and thermophilic fungi isolated on plates of glycerol asparaginate (G.A.) agar, incubated at 46°C.

i) Thermophilic actinomycetes and bacteria isolated from dairy byres.

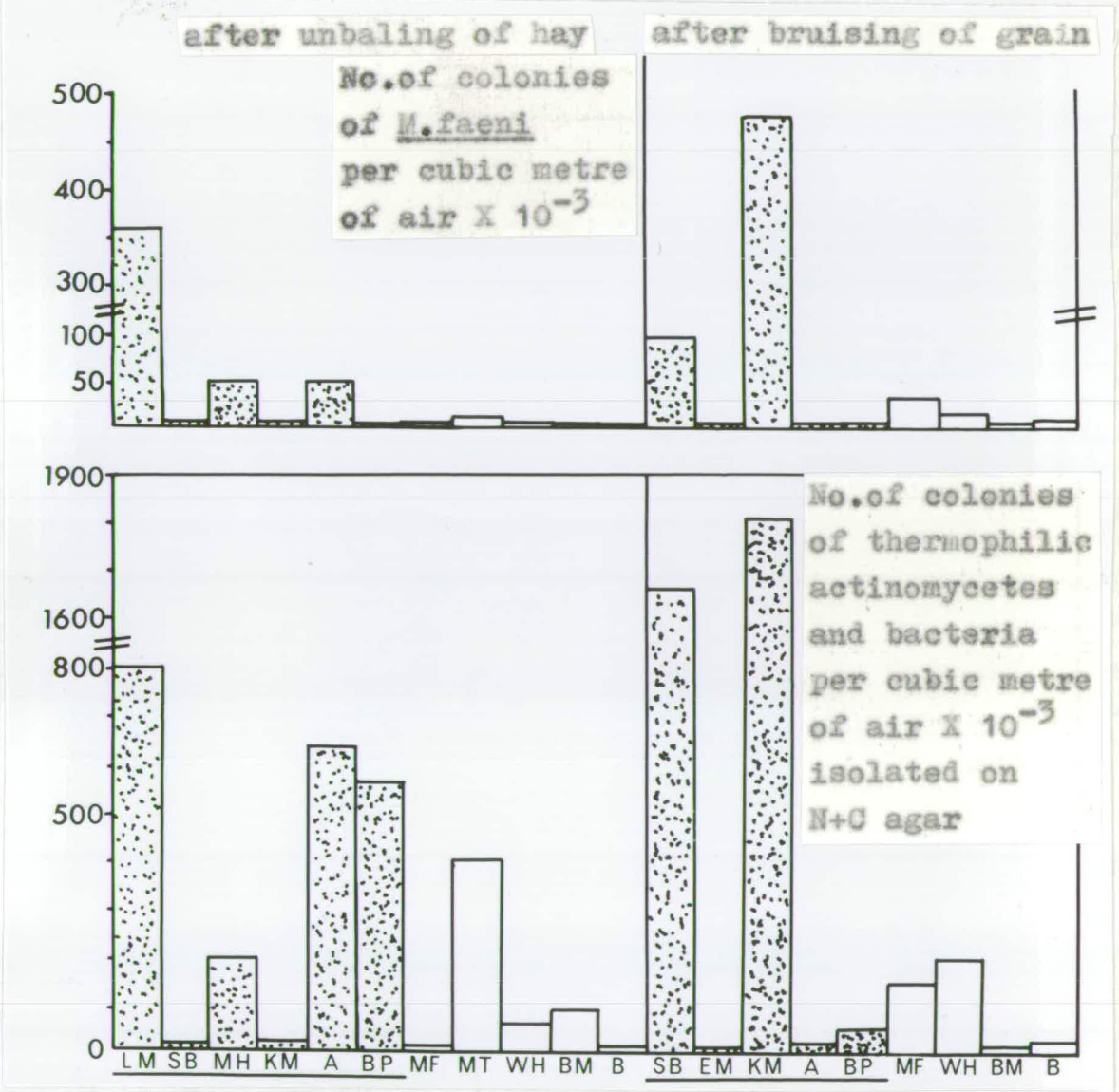
From the air of four of the eleven dairy byres sampled, the relatively high values of between 402 x 10^3 and 796 x 10^3 thermophilic bacteria and actinomycetes per cubic metre of air, were recorded (Graph 21 and Table 24). The highest value was obtained from L.M. The only non-F.L.D. farm with a high value was M.T. Apart from the dairy byre at M.H. (an FLD farm) with 190 x 10^3 colonies isolated /m^3, all other dairy byres yielded less than 100 colonies /m^3.

The majority of organisms isolated on N + C agar incubated at 46°C were actinomycetes (Table 24). On only two farms, B.M. and B., thermophilic bacteria were more common. Colonies

TYPE OF PROPAGULE	FLD FARMS					NON-FLD FARMS			
	S.B.	E.M.	K.M.	A	B.P.	M.F.	W.H.	B.M.	B
TOTAL	256	8,348	12,284	321	70	88,861	8,676	589	14,728
ASPERGILLI (GROUPS)									
A.CANDIDUS	59	1	75	6	+	548	-	-	-
A.FUMIGATUS	4	+	-	-	1	-	-	-	-
A. GLAUCUS	-	-	145	9	+	-	-	459	-
A.RESTRICTUS	-	-	-	-	-	410	-	24	1
A.TERREUS	26	-	-	1	-	-	-	11	-
A.VERSICOLOR	-	-	25	-	+	-	-	1	+
GEOTRICHUM SP	-	-	-	-	+	35,544	-	2	3
MUCORALES	5	+	-	2	1	-	-	-	-
PENICILLIUM SPP	151	8,255	11,670	282	23	7,909	7,375	12	4,562
YEASTS	5	83	369	6	14	44,403	1,301	59	10,161
OTHERS	3	-	-	5	18	-	-	23	-
ACTINOMYCETES	3	8	-	9	10	-	-	12	-

Table 23: The numbers of viable propagules ($X\ 10^{-3}$) of different species of mesophilic fungi and of mesophilic actinomycetes, cultured on malt agar, incubated at $26^{\circ}C$, per cubic metre of air from bruising sheds after bruising of grain has begun.

FLD: farmer's lung disease
NON-FLD: non-farmer's lung disease
+: less than 1 viable propagule per m^3.



Graph 21: The numbers of colonies of thermophilic actinomycetes and bacteria and those of M.faeni isolated per cubic metre of air from dairy byres after unbaling of hay and from bruising sheds after bruising of grain, on ½ strength nutrient agar containing cycloheximide(N+C) incubated at 46°C.

▨ : FLD farm
□ : non-FLD farm

TYPE OF PROPAGULE	FLD FARMS						NON-FLD FARMS				
	L.M.	S.B.	M.H.	K.M.	A	B.P.	M.F.	M.T.	W.H.	B.M.	A
TOTALS (N+C)	796	18	190	23	633	564	10	402	58	88	14
M. FAENI	308	2	51	6	45	-	3	12	4	2	1
T. VULGARIS	-	2	10	-	57	11	2	4	-	-	-
802/5	-	-	-	+	38	-	-	16	2	-	-
401	146	2	19	-	6	284	-	-	-	-	-
WHITE ACTINOMYCETES	103	+	49	5	310	40	1	358	18	11	1
BROWN ACTINOMYCETES	183	8	17	+	57	45	1	4	7	4	-
OTHER ACTINOMYCETES	-	-	13	1	6	46	-	4	11	11	+
501/2 BACTERIA	16	2	30	4	13	132	1	4	15	60	10
OTHER BACTERIA	40	+	-	7	100	6	1	-	1	-	1
THERMOPHILIC FUNGI (G.A.)	N.R.	4	14	-	457	40	40	79	26	-	6
A.FUMIGATUS		+	14	-	392	31	19	79	26	-	4
HUMICULA SP		3	-	-	-	5	21	-	-	-	-
MUCORALES		-	-	-	65	4	-	-	-	-	2
OTHERS		1	-	-	-	-	-	-	-	-	-

Table 24: The numbers of viable propagules (X 10^{-3}) of different species or groups of thermophilic actinomycetes and fungi, cultured on N+C and G.A. agars respectively, incubated at 46°C. Results are expressed per cubic metre of air for dairy byres after unbaling of hay.

FLD: farmer's lung disease

NON-FLD: non-farmer's lung disease

N+C: nutrient agar with cycloheximide

G.A.: glycerol asparaginate agar

802/5 : M. faeni variant

401 : Blue/grey/Green actinomycetes

\+ : less than 1 viable propagule per m^3

of white actinomycetes formed a large proportion of those isolated from M.H., A., M.T. and W.H., those of brown actinomycetes were common in L.M.and those of the blue/grey/green, 401-type in L.M. and B.P.

M. faeni and T.vulgaris.

The high value of 308 x 10^3 colonies of M. faeni /m^3 was recorded from L.M. and appreciable values (51/m^3 and 45/m^3) were also recorded for M.H. and A., respectively, all three being FLD farms (Table 24). Relatively few colonies were isolated from other environments. Morphologically variant colonies, designated 802/5, which were probably strains of M. faeni, were less common but values of 38 x 10^3 and 16 x 10^3 per cubic metre for these organisms coincided with counts for known M. faeni colonies of 45 x $10^3/m^3$ and 12 x $10^3/m^3$ for farms A. and M.T. Colonies of T. vulgaris were less common than those of M. faeni and the highest value was 57 x $10^3/m^3$ from farm A.

ii) Thermophilic fungi isolated from dairy byres.

In all dairy byres where thermophilic fungi were recorded, A. fumigatus (strictly a thermotolerant fungus) was the most common species (Table 24) apart from that of farm M.F., where colonies of Humicola spp. were marginally more common. Only one farm, A., which had the highest total count (457 x $10^3/m^3$) and the highest number of viable propagules of A. fumigatus (392 x $10^3/m^3$) contained appreciable numbers of propagules of thermophilic Mucorales (65 x $10^3/m^3$).

iii) Thermophilic actinomycetes and bacteria isolated from bruising sheds.

Very high values for thermophilic organisms isolated on N + C agar at 46°C were recorded for two FLD farms, S.B. and K.M. (Graph 21 and Table 25). Of the 1,664 x 10^3 organism/m^3 isolated

TYPE OF PROPAGULE	FLD FARMS					NON-FLD FARMS			
	S.B.	E.M.	K.M.	A	B.P.	M.F.	W.H.	B.M.	B
TOTAL (N+C)	1,663	12	1,811	16	46	141	193	3	21
M. FAENI	90	1	427	2	3	32	13	+	-
T. VULGARIS	-	-	-	-	2	7	1	-	-
801	6	-	-	-	-	2	1	-	-
802/5	-	-	-	2	-	70	-	-	-
401	4	1	-	-	1	-	-	-	-
WHITE ACTINOMYCETES	316	2	1,268	2	6	-	140	-	6
BROWN ACTINOMYCETES	133	-	36	-	2	11	5	+	-
OTHER ACTINOMYCETES	-	1	-	2	1	7	-	-	2
501/2 BACTERIA	1,098	5	80	7	31	5	33	2	13
OTHER BACTERIA	17	2	-	1	-	7	-	+	-
THERMOPHILIC FUNGI (G.A.)	30	14	58	3	15	2	8	-	-
ASPERGILLUS FUMIGATUS	10	5	52	1	10	1	8	-	-
HUMICOLA SPP	18	9	-	-	3	1	-	-	-
MUCORALES	2	-	-	-	2	-	-	-	-
OTHERS	-	-	6	2	-	-	-	-	-

Table 25: The numbers of viable propagules (X 10^{-3}) of different species or groups of thermophilic actinomycetes and fungi, cultured on N+C and G.A. agars, respectively, incubated at 46°C, per cubic metre of air in bruising sheds after bruising of grain has begun.

FLD: farmer's lung disease
NON-FLD: non- farmer's lung disease
N+C: nutrient agar with cycloheximide
G.A.: glycerol asparatinate agar

801: M.faeni variant?
802/5: M faeni variant
401: Blue/grey/green actinomycetes
+ : less than 1 viable propagule per m^3

from S.B., 1,098 x $10^3/m^3$ were bacteria. In K.M., white actinomycetes predominated (1,268 x $10^3/m^3$) and were also the next most common type of organism to bacteria, in S.B. Only two other farms, M.F. and W.H., had appreciable numbers of thermophilic organisms (141 x $10^3/m^3$ and 193 x $10^3/m^3$, respectively) and in the sample from the W.H. bruising-shed, white actinomycetes predominated.

M. faeni and T. vulgaris.

From the air of the bruising-shed in K.M., the highest value of 427 x $10^3/m^3$ for colonies of M. faeni isolated on N + C agar incubated at 46°C, was recorded (Table 25). The other FLD farm with high total counts, S.B., yielded 90 colones of M. faeni/m^3. From M.F., 32 colonies of M. faeni and 70 colonies of the related strain 802/5 were recorded, giving a total of 102 x $10^3/m^3$ for M. faeni type colonies. One other farm gave 13 x 10^3 colonies/m^3 but all others yielded only 3 x 10^3 colonies/m^3 or less. The concentration of T. vulgaris was negligible, this organism being isolated from only three of the nine farms at a maximum concentration of only 7 x $10^3/m^3$.

iv) Thermophilic fungi from bruising-sheds.

A.fumigatus was, as in dairy byres, the fungus most commonly isolated from G.A. plates incubated at 46°C from bruising-shdds (Table 25). Only two bruising-sheds, compared with four dairy byres, had values for the total numbers of thermophilic fungi of more than 30 colonies/m^3. Colonies of Humicola spp. were predominant in two bruising-sheds which, however, had total colony counts of only 30 x $10^3/m^3$ and 14 x $10^3/m^3$.

B. Organisms isolated from samples of hay, whole and bruised grain and dusts

a) Hay samples

i) Mesophilic fungi and actinomycetes.

The numbers of colonies of fungi and actinomycetes isolated from hay samples by the Andersen Sampler, expressed as the number per 10g. are shown in Table 26. From four FLD farms, samples of superficially 'good' and 'bad' hay from the same hay crop were collected and results for both types of hay are given. Only one 'good' sample of this group of eight hays (B.P. 44) yielded a very low count. Four other hays gave relatively low values, two from the FLD farms L.M. and S.B. and two from non-FLD farms, M.F. and W.H. A. glaucus was the species most frequently isolated from the total of five hays with low values ($8 \times 10^3/m^3$ to $229 \times 10^3/m^3$). A.restrictus and A. versicolor were the predominant species in hay sample M.T. 17 with a count of 911 colonies/10g. Eight of the fifteen hay samples released between 1,602/10g and 6,051/10g. In four of these samples (3 FLD and 1 non-FLD) A. versicolor predominated and in one (A.23), A. glaucus and A. versicolor had similarly high values. From two hay samples (one FLD and one non-FLD) A.restrictus was the species most frequently isolated and from one FLD sample, colonies of mesophilic actinomycetes predominated. The remaining hay sample, B.P. 52, realised the very high value of 17,947 colonies/10g., consisting mainly of actinomycetes and yeasts but also with high counts of A. fumigatus and members of the Mucorales. Apart from those already mentioned, only two other species of Aspergilli were isolated in appreciable numbers; A. nidulans from sample M.H. 36 and A. terreus from K.M. 34, both superficially 'bad' samples from FLD farms.

There are considerable differences in the microbiology of the 'good' and the 'bad' hay samples from FLD farms in both total counts and species isolated. This is most obvious in the difference between B.P. 44 and B.P. 52. The two most similar to one another

TYPE OF PROPAGULE	HAY SAMPLES FROM FLD FARMS										HAY SAMPLES FROM NON-FLD FARMS				
	L.M. 1	S.B. 7	M.H. 28	M.H. 36	K.M. 29	K.M. 34	A 22	A 23	B.P. 44	B.P. 52	M.F. 10	M.T. 17	W.H. 18	B.M. 27	B 38
TOTAL	71	20	3,488	4,036	3,602	2,634	1,688	6,051	8	17,947	229	911	45	3,715	1,602
ASPERGILLI (GROUPS)															
A. CANDIDUS	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-
A. FUMIGATUS	+	+	14	121	-	67	8	-	+	2,872	-	-	+	1	-
A. GLAUCUS	27	+	-	-	1	7	219	2,844	1	-	133	46	35	1	13
A. NIDULANS	+	-	-	646	-	9	-	-	-	5	5	-	-	-	-
A. RESTRICTUS	7	4	-	-	-	245	1,168	166	-	-	44	380	-	-	1,137
A. TERREUS	+	-	-	161	-	134	-	4	+	5	-	-	+	-	-
A. VERSICOLOR	-	+	3,470	2,502	3,595	3	-	2,965	-	12	23	238	-	3,566	-
AUREOBASIDIUM SP	-	-	-	-	-	21	-	-	-	59	-	-	-	-	-
CHAETOMIUM SP	+	-	-	-	-	-	-	1	-	-	-	-	-	37	-
CLADOSPORIUM SP	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-

TYPE OF PROPAGULE	HAY SAMPLES FROM FLD FARMS										HAY SAMPLES FROM NON-FLD FARMS				
	L.M. 1	S.B. 7	M.H. 28	M.H. 36	K.M. 29	K.M. 34	A 22	A 23	B.P. 44	B.P. 52	M.F. 10	M.T. 17	W.H. 18	B.M. 27	B 38
TOTAL	71	20	3,488	4,036	3,602	2,634	1,688	6,051	8	17,947	229	911	45	3,715	1,602
ASPERGILLI (GROUPS)															
A. CANDIDUS	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-
A. FUMIGATUS	+	+	14	121	-	67	8	-	+	2,872	-	-	+	1	-
A. GLAUCUS	27	+	-	-	1	7	219	2,844	1	-	133	46	35	1	13
A. NIDULANS	+	-	-	646	-	9	-	-	-	5	5	-	-	-	-
A. RESTRICTUS	7	4	-	-	-	245	1,168	166	-	-	44	380	-	-	1,137
A. TERREUS	+	-	-	161	-	134	-	4	+	5	-	-	+	-	-
A. VERSICOLOR	-	+	3,470	2,502	3,595	3	-	2,965	-	12	23	238	-	3,566	-
AUREOBASIDIUM SP	-	-	-	-	-	21	-	-	-	59	-	-	-	-	-
CHAETOMIUM SP	+	-	-	-	-	-	-	1	-	-	-	-	-	37	-
CLADOSPORIUM SP	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
FUSARIUM SP	-	-	-	-	-	-	-	-	-	36	-	-	-	-	-
GEOTRICHUM SP	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-
MUCORALES	2	-	-	4	-	45	3	1	+	2,333	-	-	+	-	-
PENICILLIUM SPP	4	+	-	444	-	5	34	12	+	21	18	9	4	74	-
PAECILOMYCES SPP	-	-	-	-	-	-	-	-	-	-	-	146	-	7	-
TRICHODERMA VIRIDE	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YEASTS	6	2	4	20	6	648	-	12	2	5,924	2	1	1	7	2
OTHERS	+	-	-	-	-	-	-	3	+	25	3	-	+	-	82
ACTINOMYCETES	4	12	-	121	-	1,050	253	42	2	6,640	+	1	2	15	368

Table 26: The numbers of viable propagules per 10 g. fresh weight of hay and the numbers of different type of propagules of mesophilic fungi and of actinomycetescultured on 2% malt agar, incubated at 26°C.

FLD: farmer's lung disease

NON-FLD: non-farmer's lung disease

+ : less than 1 viable propagule per 10 g.

in total counts and the predominance of propagules of A. versicolor, M.H. 28 and M.H. 36, differ significantly in that M.H. 36, the 'bad' sample, released propagules of A. nidulans, A. terreus and actinomycetes while M.H. 28 released none. The numbers of species released by the 'bad' hays are higher than those released by the 'good' samples from the same farm. Species present in 'bad', but absent or considerably less common in 'good' samples are A. fumigatus A. glaucus, A. nidulans, A. terreus, Mucorales, Penicillium spp., yeasts and mesophilic actinomycetes. None of the samples from non-FLD farms gave significantly high values for any of these species or groups.

ii) Thermophilic actinomycetes and bacteria from hay samples.

Four hay samples, all from FLD farms, released considerably higher numbers of propagules of actinomycetes and bacteria (more than 1,100/10g) than the other hay samples (Table 27). One of these samples, L.M.1, released very few mesophilic organisms (Table 26) but the other three, described as 'bad' as opposed to 'good' hay samples from FLD farms were those which differed most from other hay samples both qualitatively and quantitatively, in the mesophilic species released. B.P. 52 which released the largest number of mesophilic fungi also gave the highest count for thermophilic actinomycetes and bacteria (12,298/10g) (Table 27). Eight hay samples, including one 'bad' hay from an FLD farm released fewer than 40 propagules/10g and the remaining three hay samples, two from non-FLD farms and one a 'good' hay sample from an FLD farm, gave figures of 95,200 and 295 organisms/10g.

Colonies of bacteria and of the blue/grey/green 401-type formed a large proportion of those released by hays B.P. 52 and M.H. 36. The distribution of other types of actinomycetes appeared to be random apart from appreciable numbers of white colonies from

	HAY SAMPLES FROM FLD FARMS										HAY SAMPLES FROM NON-FLD FARMS				
	L.M. 1	S.B. 7	M.H. 28	M.H. 36	K.M. 29	K.M. 34	A 22	A 23	B.P. 44	B.P. 52	M.F. 10	M.T. 17	W.H. 18	B.M. 27	B 38
TOTAL (N+C)	2,959	14	13	1,107	8	2,550	21	39	249	12,298	6	95	10	200	10
M. FAENI	2,862	5	7	14	1	2,450	1	-	132	6,143	-	69	6	38	1
T. VULGARIS	-	-	+	467	5	6	-	1	27	6	-	16	+	30	3
801	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
802/5	-	1	-	-	+	8	-	2	44	-	-	-	+	-	-
401	1	1	1	208	-	6	-	-	10	1,720	-	-	-	-	-
WHITE ACTINOMYCETES	68	+	-	69	-	17	12	15	25	6	-	2	+	54	2
BROWN ACTINOMYCETES	4	5	1	135	-	25	8	15	4	2	+	4	-	78	2
OTHER ACTINOMYCETES	2	1	+	-	-	13	-	-	-	369	+	+	-	-	-
501/2 BACTERIA	21	+	2	214	1	25	-	5	7	4,052	3	3	+	+	2
OTHER BACTERIA	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-
THERMOPHILIC FUNGI (G.A.)	119	20	1	401	13	11	1	5	12	2,055	+	4	1	1	-
ASPERGILLUS FUMIGATUS	118	20	-	270	13	11	1	5	3	1,644	+	1	1	1	-
HUMICOLA SPP	-	-	1	131	-	-	-	-	-	-	-	-	-	-	-
MUCORALES	1	-	-	-	-	-	-	-	-	411	-	-	-	-	-
OTHERS	-	-	+	-	-	+	-	-	9	-	+	3	-	-	-

Table 27: The numbers of viable propagules per 10g. fresh weight of hay and the numbers of different types of propagules of thermophilic actinomycetes and fungi, cultured on N+C and G.A. agars, respectively, incubated at 46°C.

FLD: farmer's lung disease
non-FLD: non-farmer's lung disease
N+C: nutrient agar with cycloheximide
G.A.: glycerol asparaginate agar

801: M. faeni variant?
802/5: M. faeni variant
401: Blue/grey/green actinomycetes
+ : less than 1 viable propagule per 10g.

samples L.M.1, M.H.36 and B.M. 27 and of brown colonies from M.H. 36 and B.M. 27 (Table 27).

M. faeni and T. vulgaris

Very high numbers of propagules of M. faeni were released by samples L.M.1, K.M.34 and B.P.52, all from FLD farms (Table 27) Sample B.P.52 gave the highest value of 6,143 colonies of this species per 10g. Sample B.P. 44, designated a superficially 'good' hay, released 132 colonies of M. faeni per 10g. and also 44 colonies of type 802/5 per 10g. Two 'bad' hays from FLD farms released only 14 and 0 colonies of M. faeni/10g.but one of them, M.H. 36, released 467 colonies of T. vulgaris/10g. Two non-FLD hays, M.T. 17 and B.M. 27, gave appreciable values for the numbers of colonies of both M. faeni and T. vulgaris.

iii) Thermophilic fungi from hay samples.

Hay sample B.P. 52 released a high number of thermophilic fungi (2,055/10g) of which the predominant species was A. fumigatus with 411 colonies of members of the Mucorales making up the remainder (Table 27). A fumigatus predominated in the other two hay samples with relatively high values of thermophilic fungi both of which had high counts of thermophilic actinomycetes and bacteria. Humicola spp. were isolated from two samples but in appreciable numbers from one sample only(M.H. 36). Other samples, including K.M. 34, had low values for thermophilic fungi.

From these results hay samples L.M.1, M.H. 36, K.M. 34 and B.P. 52, all from FLD farms, realised high values for thermophilic organisms which were not equalled by samples from non-FLD farms. One apparently 'good' hay sample from a FLD farm gave appreciable figures, equivalent to those from two non-FLD farms and one 'bad' hay sample (A23) from an FLD farm released few colonies of either fungi or actinomycetes and bacteria.

b) Whole grain samples.

i) Mesophilic fungi and actinomycetes.

High values (2,273 to 7,076/100 g. fresh weight) were obtained for the numbers of mesophilic fungi and actinomycetes isolated on malt agar from five of the seven whole barley samples examined, three of them from FLD and two from non-FLD farms (Table 28). The whole oats sample (B.P. 48) released 509 colonies/100g. In all these samples, except K.M. 32 and B.P. 52, Penicillium spp. were predominant, followed by yeasts. A.candidus was the most common colony type from sample K.M. 32 and from sample B.P. 48, actinomycetes predominated. The remaining species or groups isolated in any concentration were, Geotrichum spp. and A. glaucus from B.42 and Mucorales and A. fumigatus from S.B.6. Four grain samples, therefore, three from FLD farms, released a microflora different from the others with high concentrations of mesophilic organisms. These were S.B.6, K.M. 32, B.P. 48 and B.42. Samples A.43 and W.H. 19 released negligible numbers of propagules of mesophilic organisms (Table 28).

ii) Thermophilic actinomycetes and bacteria isolated from whole grain samples.

The greatest numbers of colonies of thermophilic actinomycetes isolated from whole grain samples were from S.B.6 and K.M.32, both FLD farms (Table 29). Appreciable numbers were released by two samples from FLD farms and two from non-FLD farms. The remaining two samples, A.43 and M.F.11, released negligible values.

Colonies of white actinomycetes were relatively commonly isolated from S.B.6 and K.M. 32 and brown actinomycetes from B.P. 48. Bacteria of the 501/2 type were also present in appreciable numbers, from E.M. 14.

	WHOLE BARLEY SAMPLES FROM FLD FARMS					SAMPLES FROM NON-FLD FARMS		
TYPE OF PROPAGULE	S.B. 6	E.M. 14	K.M. 32	A 43	B.P. 48(O)	M.F. 11	W.H. 19	B 42
TOTAL	5,359	7,076	2,556	28	509	5,796	5	2,273
ASPERGILLI (GROUPS)								
A.CANDIDUS	-	-	1,917	1	8	-	-	-
A. FUMIGATUS	61	-	-	-	2	-	-	-
A. GLAUCUS	-	-	3	4	1	-	-	159
A. NIGER	+	-	-	-	-	-	-	-
A. RESTRICTUS	-	-	-	+	-	-	-	-
A.TERREUS	+	-	-	+	-	-	-	-
A.VERSICOLOR	-	-	8	+	17	-	-	-
CHAETOMIUM SPP	9	-	-	-	-	-	+	-
CHRYSOSPORIUM BPP	-	-	-	+	+	-	-	-
GEOTRICHUM SP	-	-	-	+	-	-	-	591
MUCORALES	112	-	3	2	36	-	-	-
PAECILOMYCES SPP	4	-	-	+	-	-	-	-
PENICILLIUM SPP	4,770	4,458	26	4	316	3,767	+	1,023
YEASTS	268	2,547	37	10	97	2,029	4	432
OTHERS	-	-	-	-	-	-	+	68
ACTINOMYCETES	135	-	5	31	562	+	-	-

Table 28: The numbers of viable propagules per 100g. fresh weight of whole barley and oats (O) and the numbers of different types of propagules of mesophilic fungi and of actinomycetes cultured on 2% malt agar, incubated at 26°C.

FLD: farmer's lung disease
NON-FLD: non- farmer's lung disease
+ : less than 1 viable propagule per 100g.

	WHOLE BARLEY SAMPLES FLD FARMS					WHOLE BARLEY SAMPLES NON-FLD FARMS		
	S.B. 6	E.M. 14	K.M. 32	A 43	B.P. 48(O)	M.F. 11	W.H. 19	B 42
TOTAL (N+C)	802	271	2,334	5	179	8	125	160
M. FAENI	618	160	1,821	1	13	-	33	46
T. VULGARIS	-	2	+	+	6	-	+	1
801	1	-	-	-	-	-	27	-
802/5	80	2	210	+	13	-	5	-
401	6	33	+	-	1	6	14	+
WHITE ACTINOMYCETES	64	5	257	1	27	+	5	2
BROWN ACTINOMYCETES	24	+	3	-	79	+	14	22
OTHER ACTINOMYCETES	-	-	+	+	13	+	6	85
501/2 BACTERIA	6	68	41	2	27	+	19	3
OTHER BACTERIA	3	-	-	-	-	-	2	+
THERMOPHILIC FUNGI (GA)	4	9	9	1	46	-	59	-
ASPERGILLUS FUMIGATUS	2	3	5	1	42	-	3	-
HUMICOLA SPP	-	2	1	-	-	-	-	-
MUCORALES	2	2	3	-	4	-	1	-
OTHERS	+	2	-	-	-	-	55	-

Table 29: The numbers of viable propagules per 100g. fresh weight of whole barley and oats (O), and the numbers of different types of propagules of thermophilic actinomycetes and fungi, cultured on N+C and G.A. agars, respectively, incubated at 46°C.

FLD: farmer's lung disease
NON-FLD: non- farmer's lung disease
N+C: nutrient agar with cycloheximide
G.A.: glycerol asparaginate agar

801: M faeni variant ?
802/5: M faeni variant
401: Blue/grey/green actinomycetes
+ : less than 1 viable propagule per 100 g.

M. faeni and T. vulgaris.

Colonies of M. faeni formed the largest proportion of thermophilic actinomycetes isolated from S.B. 6 and K.M. 32 (802/100 g and 2,334/100 g. respectively)(Table 29). Colonies of the 802/5 type were also isolated in appreciable numbers from these two samples. Sample E.M. 14, another from an FLD farm, also released appreciable numbers of propagules of M. faeni (160/100 g.) as did W.H. 19 and B.42, from non-FLD farms with values of 33/100 g. and 46/100 g. respectively. T. vulgaris was isolated only in small concentrations from six of the eight whole grain samples.

iii) Thermophilic fungi from whole grain samples

Samples B.P. 48 and W.H. 19 released 46 and 59 thermophilic fungi per 100 g. respectively (Table 29). A. fumigatus predominated from B.P. 48. Other samples yielded few or no colonies of thermophilic fungi/100g.

c) Bruised grain samples.

i) Mesophilic fungi, actinomycetes and bacteria.

Five of the six processed grain samples, two from FLD farms and three from non-FLD farms released between 1,679 and 11,781 mesophilic organisms per 100 g. (Table 30). The highest value was from S.B.5 and consisted of colonies of Pencillium spp. with the exception of two colonies of A. terreus. Colonies of Pencillium spp. formed a large proportion of those released by M.F.16 and B.40, as did colonies of Geotrichum sp. Yeasts predominated from B.M. 30 and bacteria from A.41. Bacteria also formed a large proportion of the colonies from B.40. A. candidus, Paecilomyces spp. and A. versicolor were recorded in appreciable numbers from A.41, M.F.16 and B.40, respectively.

ii) Thermophilic actinomycetes and bacteria from bruised grain samples.

Only one sample, S.B. 5, released large numbers of thermophilic

	BRUISED BARLEY SAMPLES FROM FLD FARMS			BRUISED BARLEY SAMPLES FROM NON-FLD FARMS		
TYPE OF PROPAGULE	S.B. 5	E.M. 15	A 41	M.F. 16	B.M. 30	B 40
TOTAL	11,781	21	2,250	3,384	1,679	4,355
ASPERGILLI (GROUPS)						
A. CANDIDUS	-	+	56	-	-	-
A. FUMIGATUS	-	+	+	-	-	-
A. GLAUCUS	-	+	10	-	-	23
A. RESTRICTUS	-	-	-	-	-	3
A. TERREUS	2	-	+	-	-	-
A. VERSICOLOR	-	-	-	-	-	136
AUREOBASIDIUM SPP	-	-	-	-	21	-
CLADOSPORIUM SP	-	-	-	-	27	-
GEOTRICHUM SP	-	6	1	1,319	-	1,655
PAECILOMYCES SPP	-	-	-	474	-	-
PENICILLIUM SPP	11,779	7	32	1,589	39	1,350
YEASTS	-	8	2	-	1,561	-
OTHERS	-	-	3	-	24	12
ACTINOMYCETES	-	-	1	2	7	-
BACTERIA			2,138			1,176

Table 30: The numbers of viable propagules per 100g. fresh weight of bruised barley and the numbers of different types of propagules of mesophilic fungi and of actinomycetes cultured on 2% malt agar, incubated at 26°C.

FLD: farmer's lung disease

NON-FLD: non-farmer's lung disease

+ : less than 1 viable propagule per 100 g.

actinomycetes and bacteria and of these, colonies of 801 (strains of M. faeni?) predominated (Table 31). One other sample, A.41, released appreciable numbers of thermophilic actinomycetes and bacteria of which bacteria of type 501/2 predominated.

M. faeni and T. vulgaris

Forty colonies of M. faeni/100 g. were isolated from A.41 and 23 colonies of 802/5 type were isolated per 100 g. of sample S.B.5 (Table 31). These two samples from FLD farms, therefore, contained M. faeni strains as well as relatively small numbers of colonies of T. vulgaris.

iii) Thermophilic fungi from bruised grain samples.

Only one processed barley, S.B.5., from an FLD farm, released thermophilic fungi, of which A. fumigatus was the most common species (Table 31).

d) Dust samples from bruising machines.

i) Mesophilic fungi and actinomycetes.

Three dust samples, S.B.3., W.H.20 and B.37 released large numbers of mesophilic fungi (Table 32). Colonies of Penicillium spp. predominated from W.H. 20 but yeasts were more common than Penicillium spp.from both S.B.3 and B.37.

A. candidus and A. terreus from S.B. 3, actinomycetes from S.B.3 and K.M. 31 and a Geotrichum sp. from M.F.12 were the only other species or groups of mesophilic organisms present in appreciable numbers.

ii) Thermophilic actinomycetes and bacteria from dust samples.

Two dust samples from FLD farms (Table 33) S.B.3 and E.M.13 released relatively high numbers of thermophilic actinomycetes and bacteria (430/10g and 239/10g. respectively). Of these, colonies of white actinomycetes predominated from S.B.3 and colonies of bacteria 501/2, from E.M. 13. The moderate number of 46 colonies/

	BRUISED BARLEY SAMPLES FLD FARMS			BRUISED BARLEY SAMPLES NON-FLD FARMS		
TYPE OF PROPAGULE	S.B. 5	E.M. 15	A. 41	M.F. 16	B.M. 30	B. 40
TOTAL	1,576	3	132	1	3	21
M. FAENI	-	-	40	-	-	-
T. VULGARIS	13	-	4	-	-	1
801	1,182	-	3	-	-	-
802/5	23	-	4	-	-	-
401	-	-	-	-	-	-
WHITE ACTINOMYCETES	173	2	11	-	-	-
BROWN ACTINOMYCETES	79	-	+	-	-	-
OTHER ACTINOMYCETES	74	-	-	-	-	-
501/2 BACTERIA	22	1	70	1	3	20
OTHER BACTERIA	10	-	-	-	-	-
THERMOPHILIC FUNGI (G.A.)	31	-	-	-	-	-
ASPERGILLUS FUMIGATUS	21	-	-	-	-	-
MUCORALES	1	-	-	-	-	-
OTHERS	9	-	-	-	-	-

Table 31: The numbers of viable propagules per 100 g. fresh weight of bruised barley and the numbers of different types of propagules of thermophilic actinomycetes and fungi, cultured on N+C and G.A. agars, respectively, incubated at 46°C.

FLD: farmer's lung disease

NON-FLD: non-farmer's lung disease

N+C: nutrient agar containing cycloheximide

G.A.: glycerol asparaginate agar

801: M. faeni variant?

802/5: M. faeni variant

401: Blue/grey/green actinomycetes

+ : less than 1 viable propagule per 100 g.

TYPE OF PROPAGULE	DUST SAMPLES FROM FLD FARMS					DUST SAMPLES FROM NON-FLD FARMS		
	S.B. 3	E.M. 13	K.M. 31	A. 24	B.P. 46	M.F. 12	W.H. 20	B 37
TOTAL	1,689	2	54	28	2	16	847	6,713
ASPERGILLI (GROUPS)								
A.CANDIDUS	34	-	-	+	-	+	-	4
A. FUMIGATUS	3	-	+	+	+	-	-	-
A. GLAUCUS	-	+	-	4	-	1	-	-
A. TERREUS	16	-	1	-	-	-	-	-
A. VERSICOLOR	2	-	+	-	-	+	1	-
GEOTRICHUM SP	+	-	-	+	-	14	-	833
MUCORALES	-	-	-	-	+	-	-	-
PAECILOMYCES SPP	-	+	1	-	-	-	8	-
PENICILLIUM SPP	29	+	2	14	1	+	734	2,385
YEASTS	1,436	-	2	2	-	+	131	3,491
ACTINOMYCETES	169	+	46	6	+	+	+	-

Table 32: The numbers of viable propagules per 10 g. of dust off bruising machines and the numbers of different types of propagules of mesophilic fungi and of actinomycetes cultured on 2% malt agar, incubated at 26°C.

FLD: famer's lung disease
NON-FLD: non-farmer's lung disease
+ : less than 1 viable propagule per 10g.

	DUST SAMPLES FROM FLD FARMS					DUST SAMPLES FROM NON-FLD FARMS		
TYPE OF PROPAGULE	S.B. 3	E.M. 13	K.M. 31	A 24	B.P. 46	M.F. 12	W.H. 20	B 37
TOTAL (N+C)	430	239	46	20	6	26	2	27
M. FAENI	73	-	28	1	-	-	+	4
T. VULGARIS	-	-	-	1	+	-	-	4
801	-	-	-	-	-	-	-	-
802/5	4	-	10	1	+	4	+	-
401	-	-	-	-	-	-	-	3
WHITE ACTINOMYCETES	288	3	7	1	+	-	+	4
BROWN ACTINOMYCETES	30	-	-	7	3	-	-	-
OTHER ACTINOMYCETES	9	2	-	-	-	4	+	-
501/2 BACTERIA	22	234	1	8	2	18	1	12
OTHER BACTERIA	4	-	-	+	-	-	-	-
THERMOPHILIC FUNGI (G.A.)	1	-	1	-	2	-	-	2
ASPERGILLUS FUMIGATUS	1	-	+	-	+	-	-	2
HUMICOLA SPP	-	-	+	-	-	-	-	-
MUCORALES	-	-	+	-	1	-	-	-
OTHERS	-	-	+	-	+	-	-	-

Table 33: The numbers of viable propagules per 10 g. of dust off bruising machines and the numbers of different types of propagules of thermophili actinomycetes and fungi, cultured on N+C and G.A. agars, respectively, incubated at 46°C.

FLD: farmer's lung disease

NON-FLD: non-farmer's lung disease

N+C: nutrient agar containing cycloheximide

G.A.: glycerol asparaginate agar

801: M. faeni variant ?

802/5: M. faeni variant

401: Blue/grey/green actinomycetes

+ : less than 1 viable propagule per 10g.

10g. was released by K.M. 31, three samples with predominantly bacterial colonies released between 20 and 27 colonies/10g. and the remaining two samples yielded only 6 and 2 colonies /10g.

M. faeni and T. vulgaris

M. faeni was isolated in appreciable numbers from S.B.3 and K.M. 31 (73 and 28 colonies per 10 g. respectively; Table 33). Colonies of 802/5 were also isolated from these two samples and from M.F.12, but in smaller concentrations (4 to 10 colonies /10g.). M. faeni and T. vulgaris were both isolated in small numbers (4 colonies/10g.) from B.37.

iii) Thermophilic fungi from dust samples.

Only 0, 1 or 2 colonies of thermophilic fungi/10g. were isolated from four of the eight dust samples examined. A. fumigatus was the species most frequently isolated (Table 33).

The numbers of propagules of M. faeni per cubic metre of air after unbaling of hay or bruising of grain in a farm building compared with the numbers released by samples of hay and grain from the same farm.

Results obtained for the numbers of colonies of M. faeni, per litre of air, isolated on N + C agar incubated at 46°C from farm buildings after unbaling of hay or bruising of grain are compared with values obtained per 1g. of hay or per 10g. of whole barley from the respective farms in Graphs 22a and 22b. Of six FLD farms, five realised very high or appreciable values for the numbers of colonies of M. faeni per 1g. of hay or per cubic metre of air in the dairy byre. Farm S.B. was the only FLD farm with low counts for both. Farm M.T. was the only non-FLD farm with moderate numbers of M. faeni per cubic metre of air in the dairy byre.

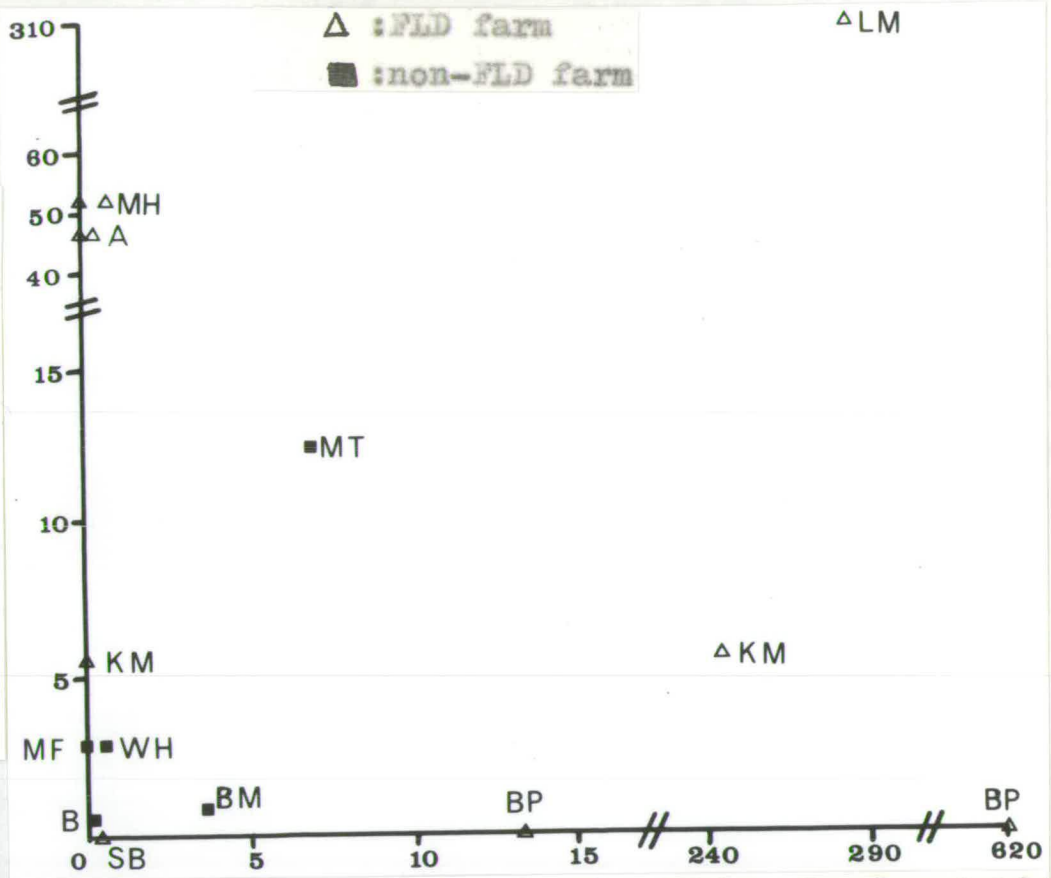
Farms K.M. and S.B., both FLD farms, gave high values for the numbers of propagules of M. faeni collected per cubic metre of air after bruising

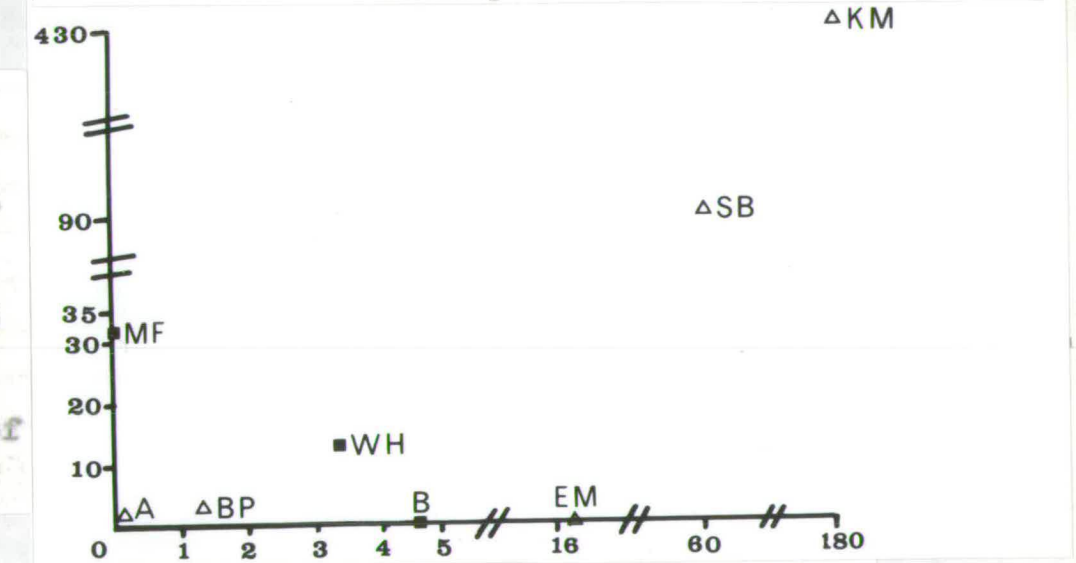
of grain and also per 10g. of their whole barley samples. Farm E.M., also a FLD farm, released appreciable numbers of M. faeni/10g. of whole barley compared with barley samples other than those of K.M. and S.B. Appreciable numbers of M. faeni propagules were collected from the air of non-FLD farm M.F., after bruising of grain.

From colonies grown on N + C agar, incubated at 46°C, it would appear that FLD farms have a considerably higher concentration of M. faeni than non-FLD farms in either the numbers of propagules isolated from the air or those from samples of hay and grain.

The situation is, however, less simple when the results of air samples obtained from N + C agar plates incubated at 60°C are taken into consideration (Tables 20 and 21). Farms L.M., M.H., K.M. and S.B. (all FLD) gave values of between 1,193 x $10^3/m^3$ and 2,020/m^3 colonies of M. faeni isolated on N + C agar incubated at 46°C or 60°C after unbaling of hay or bruising of grain. The remaining three FLD farms and four of the five non-FLD farms do not approach this value but from non-FLD farm M.F., 1,052 colonies of type 801 were collected per cubic metre of air after incubation of N + C plates at 60°C as well as appreciable numbers of colonies (52 x $10^3/m^3$) classified as M. faeni.

On the basis of numbers of M. faeni propagules from hay and grain, samples from FLD farms L.M., K.M., S.B. and B.P. (Graph 22) gave significantly higher values than samples from other farms. Therefore, five of seven FLD farms (excluding E.M. and A.) realised very high concentrations of M. faeni either from the air of farm buildings or from samples of hay or grain. Farm M.F. is the only non-FLD farm with high counts of what may be M. faeni from the dairy byre after unbaling of hay. Otherwise, the maximum values for the numbers of colonies of M. faeni and 802/5 type per cubic metre of air, after unbaling of hay or bruising of grain from FLD farms E.M. and A. and non-FLD farms M.F. and M.T. are 37 x 10^3, 83 x 10^3, 102 x 10^3 and 62 x 10^3, respectively. The highest





Graph 22: The number of colonies of M.faeni isolated per cubic metre of air from dairy byres after unbaling of hay and from bruising sheds after bruising of grain, compared with the numbers of colonies of M.faeni isolated per 1g. fresh weight of hay and 10g. fresh weight of grain respectively on farmer's lung disease (FLD) and non-FLD farms.

equivalent values for non-FLD farms W.H., B.M. and B were 13×10^3, 2×10^3 and 3×10^3, respectively.

Conclusion

1. Methods of harvesting and storing hay were similar on the farms studied, as was harvesting of barley. Storage of barley and oats was by several methods, utilising artificial drying, addition of propionic acid as preservative or exclusion of air from the grain by the use of polythene bags or a sealed silo, resulting in an accummulation of inhibitory concentrations of carbon dioxide.

2. With few exceptions, values obtained for the total numbers of mesophilic fungi and actinomycetes and of thermophilic actinomycetes and bacteria were considerably higher in farm buildings after unbaling of hay and bruising of grain than before these activities.

3. The highest proportion of mesophilic colonies isolated after unbaling of hay and bruising of grain was of fungi isolated on plates 0 to 3 of the Andersen Sampler with the exception of farm A, after unbaling of hay, when colonies of mesophilic actinomycetes predominated. It was, therefore, considered valid that the numbers of colonies isolated on the upper four plates of the Andersen Sampler gave an estimate of the relative proportions of mesophilic fungi in different environments.

4. Plates 4 to 7 of the eight-stage Andersen Sampler gave an estimate of the numbers of colonies of thermophilic actinomycetes and bacteria in the atmosphere as the majority of colonies of these organisms were collected on the lower four plates of the Sampler.

5. The gravity Petri-dish technique was not as efficient as the Andersen Sampler in either qualitative or quantitive terms i.e. in either the total numbers or the numbers of different types of colonies isolated. The G.P.D. technique was more efficient in isolating different colony types of fungi than it was in isolating colony types of actinomycetes or bacteria.

6. Nutrient agar containing cycloheximide was more efficient than glycerol asparaginate agar in allowing growth of thermophilic actinomycetes and bacteria but G.A. agar had the advantage of allowing growth

of thermophilic fungi.

7. The numbers of thermophilic actinomycetes and bacteria and particularly the numbers of colonies of Micropolyspara faeni isolated from farm buildings were generally higher from plates of N + C agar incubated at 60°C than from those incubated at 46°C. In general, a larger number of thermophilic fungi was isolated from farm buildings on plates of G.A. agar incubated at 46°C than on those incubated at 60°C.

8. There was no distinction between FLD and non-FLD farms in the numbers of mesophilic colonies isolated from dairy byres or bruising sheds and a wide range of values was obtained from both types of building.

9. The highest proportion of mesophilic organisms isolated from dairy byres consisted of members of the genus Aspergillus and from bruising sheds, of members of the genus Penicillium and yeasts.

10. The highest concentration of mesophilic organisms from the air of dairy byres was 5,418 x $10^3/m^3$, obtained from FLD farm B.P. and consisted mainly of colonies of A. fumigatus, A. nidulans, Mucorales and yeasts which were isolated in low concentrations, if at all, from other dairy byres. The highest concentration of mesophilic organisms from bruising sheds was 88,861 x $10^3/m^3$, obtained from a non-FLD farm, M.F., and consisted mainly of colonies of yeasts and a Geotrichum sp. with Penicillium spp. and appreciable numbers of A. candidus.

11. The numbers of thermophilic actinomycetes and bacteria were high (between 564 x $10^3/m^3$ and 1,670 x $10^3/m^3$) in four of the six dairy byres from FLD farms but in only two of five non-FLD farms. Only two FLD farms, S.B. and K.M. gave high concentrations of these organisms from bruising sheds, with maximum values of 1,664 and 2,244 x $10^3/m^3$, respectively.

12. Of the thermophilic actinomycetes isolated, M. faeni formed a large proportion of those from four FLD farms, giving values of between 1,193 x 10^3 and 2,020 x 10^3 colonies/m^3 from air of two dairy byres and two

bruising sheds. Concentrations of this organism differentiated most clearly between FLD and non-FLD farms although three FLD farms yielded low concentrations of M. faeni from their buildings and one non-FLD farm yielded a concentration of $1,052/m^3$ of colony type 801 which may have been a M. faeni variant.

13. T. vulgaris was less commonly isolated and in much smaller concentrations than M. faeni. The maximum concentration of T. vulgaris was $110 \times 10^3/m^3$ from FLD farm B.P., after unbaling of hay.

14. The concentration of thermophilic fungi isolated from farm buildings was low, the highest value of $457 \times 10^3/m^3$ from FLD farm, A., after unbaling of hay, being an exception, as the majority of values were less than 40×10^3 colonies/m^3.

15. Organisms isolated from samples of hay, grain and dust indicated that, as was the case for the air of dairy byres and bruising sheds, differentiation between FLD and non-FLD farms was most clearly demonstrated by high concentrations of certain species of mesophilic fungi and also of thermophilic organisms, particularly of the thermophilic actinomycete, M.faeni from FLD farms. The following observations substantiate the statement above:

i) There was little differentiation between samples of hay from FLD farms and non-FLD farms or between 'good' and 'bad' hay samples from the same FLD farm on the basis of the numbers of mesophilic organisms released per unit weight. The fungal species most frequently isolated from hay samples were, as for dairy byres, members of the genus Aspergillus. There was a wider range of fungal species isolated from 'bad' hays than from 'good' hays from the same FLD farms or from samples from non-FLD farms. The numbers of the following species per unit weight were higher from samples of 'bad' hays from FLD farms than from other samples: A fumigatus, A.glaucus, A.nidulans, A. terreus, Mucorales, Penicillium spp., yeasts and mesophilic actinomycetes. The 'bad' hay sample from

FLD farm B.P., which released the highest number of propagules of mesophilic organisms (17,947/10g) released predominantly mesophilic actinomycetes, yeasts and also relatively high numbers of propagules of A. fumigatus and Mucorales.

ii) 'Bad' hay samples from FLD farms, with only one exception, realised considerably higher numbers of thermophilic actinomycetes and bacteria than either 'good' hay samples from FLD farms or samples from non-FLD farms. Four hay samples from FLD farms yielded very high numbers of thermophilic actinomycetes and bacteria per 10 g. and from three of these samples the concentration of propagules of M. faeni was between 2,450/10g. and 6,143/10g. Only three other samples, one a 'good' hay sample from an FLD farm and two from non-FLD farms yielded appreciable numbers of thermophilic actinomycetes and bacteria, including colonies of M. faeni. The 'bad' hay sample with the highest concentration of mesophilic fungi and actinomycetes released the highest numbers of thermophilic actinomycetes and bacteria and also of thermophilic fungi (2,055/10g.), making a total count of 32,300 propagules per 10g. Of the remaining fourteen hay samples only two released appreciable numbers of thermophilic fungi and the remainder released less than 20 colonies per 10g.

iii) There was no differentiation between samples from FLD and those from non-FLD farms on the basis of the numbers of mesophilic colonies isolated per unit weight of whole or processed grain or of dust from bruising-machines. The highest numbers of mesophilic propagules from whole barley were 7,076/100g., from bruised barley were 11,781/100g. and from dust were 6,713/100g. The colonies most commonly isolated from these types of sample were similar to those isolated from the air of bruising sheds, as Penicillium spp. yeasts and Geotrichum spp. were most commonly isolated from whole and bruised

grain and Penicillium spp.and yeasts were most commonly isolated from dust samples. Bruised barleys were the only samples to release large concentrations of mesophilic bacteria, A.candidus was recovered from the whole barley sample from FLD farm, K.M., at the high concentration of 1,917/100g. and appreciable numbers of colonies of A. fumigatus and Mucorales were recovered from the whole barley sample from FLD farm, S.B.

iv) Whole barley samples from FLD farms S.B. and K.M., besides releasing the species of mesophilic fungi in (iii) above gave the highest numbers of colonies of thermophilic actinomycetes and bacteria and particularly of M. faeni per 100 g. The value for the K.M. sample was 1,821 colonies of M. faeni per 100g. The S.B. bruised barley sample released 1,182 colonies of the 801 type (a M. faeni variant?) and dusts only from K.M. and S.B. released appreciable numbers of thermophilic actinomycetes and bacteria, including M. faeni per 10g. Apart from 160 colonies of M. faeni isolated from one other whole barley sample (from FLD farm E.M.) there was no other marked differentiation between FLD and non-FLD farms with respect to the thermophilic organisms isolated from whole and bruised grain and dust.

v) On three FLD farms, L.M., M.H. and B.P., there was evidence of high numbers of thermophilic actinomycetes and bacteria particularly of M. faeni from the air of dairy byres and/or hay samples. On two FLD farms, K.M. and S.B., the air of the bruising sheds after bruising of grain and/or samples of whole or bruised grain and dust off bruising-machines contained large numbers of thermophilic actinomycetes and bacteria, notably M. faeni. The remaining two FLD farms, E.M. and A. realised moderate numbers of colonies of thermophilic actinomycetes and bacteria, including M. faeni,as did the non-FLD farms, M.T. and M.F. After unbaling

of hay in M.F., however, large numbers of colonies of type 801 (a variant of M. faeni?) were released. The other three non-FLD farms realised small concentrations of thermophilic actinomycetes and bacteria from the air of farm buildings or from samples of hay, grain or dust.

SECTION IV

THE SEROLOGY OF SELECTED FARMING PERSONNEL IN AYRSHIRE WITH REFERENCE TO FARMER'S LUNG DISEASE.

The Serology of Selected Farming Personnel in Ayrshire with reference to Farmer's Lung Disease.

Materials and Methods.

1. Sources of Sera.

Sera were collected from personnel on nine Ayrshire dairy farms, each with a medically well-documented case of farmer's lung disease (FLD farms) and from five farms with no record of farmer's lung disease in the six years previous to 1972 (non-FLD farms). Twenty ml. blood samples were taken from farmers, farm-workers and farmer's wives actively involved in farm work by clinicians from the Respiratory Diseases Unit, Northern General Hospital, Edinburgh. These samples were centrifuged at 5,000 r.p.m. for 20 minutes on the evening of the day they were taken. The sera were pipetted off and sodium azide was added to a final concentration of 0.02% before storage at $4^{o}C$. Two FLD sera, one from a patient in Perthshire (B) and one from Ayrshire (C) were collected when these farmers attended hospital as outpatients. The samples used for serological testing came from a total of eleven medically well-documented cases of FLD, fourteen non-FLD personnel on FLD farms and thirteen non-FLD personnel on non-FLD farms. A control group of fifty sera were obtained from the Blood Transfusion Service.

2) Sources of Antigen.

Antigens from Fungi and Actinomycetes

Culture filtrate (CF) and mycelial (M) antigens were obtained from shake cultures of the fungi and actinomycetes most commonly found on both FLD and non-FLD farms. Extracts were made from twelve mesophilic fungi, from four thermotolerant or thermophilic fungi, from sixteen thermophilic actinomycetes and also from two thermophilic bacteria. Mesophilic fungi, were Rhizopus sp., Cladosporium sp., Geotrichum sp., Pencillium sp. and species from the following groups of Aspergilli, A. fumigatus, A. terreus, A. glaucus (2 strains).

A. nidulans, A. candidus, A. versicolor and A. restrictus. Thermotolerant fungi were two strains of species of the A. fumigatus group and thermophilic fungi were a Humicola sp.and a Mucor sp. Thermophilic actinomycetes consisted of six strains of Micropolyspora faeni isolated from different farms, four strains of M. faeni variant (802/5) five strains of Streptomyces spp.,one strain of actinomycete type 401 (page 115) and one strain of Thermoactinomyces vulgaris.

Antigens were prepared from type cultures of M. faeni and T. vulgaris provided by Dr. J. Lacey, Rothamsted Experimental Station, Harpenden, Herts.

Culture Conditions for Fungi and Actinomycetes.

a) Mesophilic fungi

Inocula of mesophilic fungi were added to aliquots of 400 ml. Czapek Dox liquid medium (Oxoid) containing 2g. Nutrient Broth (Oxoid) in 1 litre conical flasks. The culture flasks were then shaken for six weeks in an orbital incubator at 26°C in darkness.

b) Thermophilic and thermotolerant fungi

Thermophilic and thermotolerant fungi were cultured in the same medium as mesophilic fungi but were grown in the incubator at 46°C for only three weeks.

c) Thermophilic actinomycetes

Inocula of thermophilic actinomycetes were added to 400 ml. aliquots of Nutrient Broth (Oxoid) containing 1% glucose in 1 litre conical flasks and shaken for three weeks in an orbital incubator, at 46°C.

Antigen Extraction from Fungi and Actinomycetes

Culture filtrate (CF) and mycelial (M) antigens from fungi and actinomycetes were extracted from the culture broths and mycelial mats of the organisms concerned after the requisite growth period, according to the flow chart in Figure 21.

II Antigens from Samples of Hay, Grain and Dust

Antigens were extracted from samples of hay, from whole and processed grain and from dusts collected from bruising or milling machines. All types of sample were from both FLD and non-FLD farms.

a) Extraction of antigens using Coca's Solution

Samples of 5g. of hay or dust and of 10g. of whole or processed grain were placed in 1 litre screw-topped jars. The samples were defatted in 200 ml. acetone overnight and after decantation the acetone was passed through three layers of muslin to retain any sizeable pieces of material. 200 ml. of Coca's solution (Appendix C) were added to each of the samples and the jars were shaken for seven days in an orbital incubator at 26°C. The resulting solutions were filtered through three layers of muslin and a sintered-glass funnel, Porosity 2. The debris of the samples was discarded and the solutions were dialysed for seventy-two hours against running tap water, concentrated against polyethylene glycol 4000 and dried in a rotary film evaporator. The dried powder was reconstituted to a concentration of 50-60 mg/ml by addition of distilled water. This was used in serological tests at a strength of 20-25 mg./ml.

b) Extraction of antigens using Trichloracetic Acid (T.C.A.)

Five gram samples of hay or dust and 10 gram samples of whole or processed grain were placed in 1 litre screw-topped jars. To each of these samples was added 200 ml. 5% T.C.A. and they were then shaken for three days in an orbital incubator at 26°C. Further processing was as described above for extraction using Coca's solution.

3) Serological Tests

a) Double diffusion in agar

Double diffusion (DD) in agar was carried out as described by Ouchterlony (1953). Molten, 1.5% veronal buffered agar, pH 8.2 (Appendix C) was poured to a thickness of 2 mm onto glass plates resting on a levelling table. A central serum well of 4mm diameter was surrounded by five peripheral antigen wells of 3 mm diameter. Margins of wells

containing reactants were 4 mm apart. The test plates were incubated for a minimum of five days in a humid atmosphere at 26°C.

b) Immunoelectrophoresis

Immunoelectrophoresis (IE) was carried out on glass plates coated with a 2mm thickness of 1.5% agar in half-strength veronal buffer, pH 8.2 (Appendix C). Full strength buffer was used in the Kohn electrophoresis tank (Shandon). Double thickness, 3MM chromatography paper formed the wicks between agar and buffer. Antigens were placed in wells of 2mm diameter, 1.2 cm apart (Figure 22) and constituents were separated at 150 V for 1 h.45 mins. Antiserum troughs (1 mm x 5cm) were cut midway between the antigen walls (Figure 22), the cathodal ends of the troughs being 1.5 cm from the antigen wells, as recommended by Pepys and Jenkins, 1965. Reactions were allowed to develop in a humid atmosphere at 26°C for 72 hours.

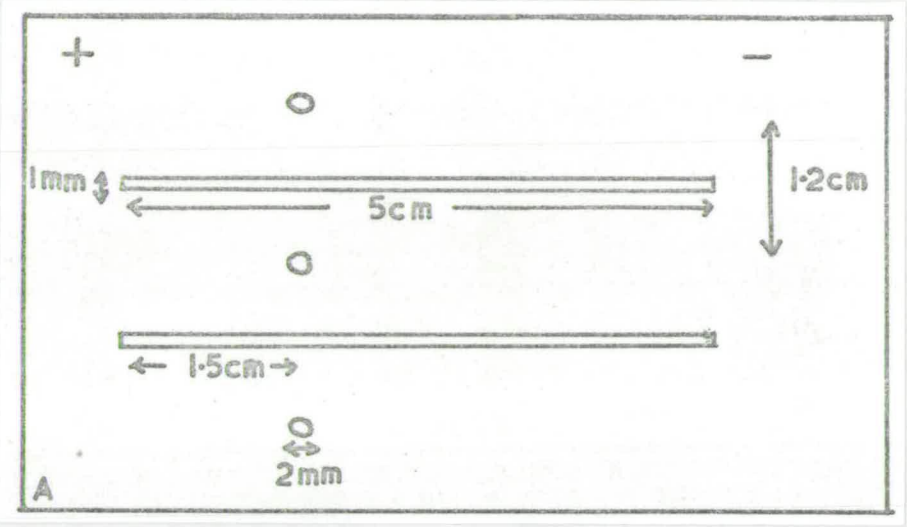
c) Immunoelectroosmophoresis

Immunoelectroosmophoresis(I.E.O.) was carried out in the same agar and buffer system as that for I.E. The contents of opposing antigen and antiserum wells, 3 mm diameter and 5 mm apart, with 10 mm between rows, (Gordon et al., 1971: Figure 22) were allowed to interact for 2h. at 100 - 150 V, with the antiserum well adjoining the cathode. Plates were then washed, dried and stained.

4) Washing, Drying and Staining of Gels used in Serological Tests

i) Washing of agar gels

Agar gels from the D.D. test were placed in 5% sodium citrate, pH 8.2 (Longbottom and Pepys, 1964) for six to eight hours to remove non-specific precipitate due to C-substance. Serum and antigenic material which had diffused into the gel but was not bound in the precipitin arcs was leached out by placing gels from the D.D. test in daily changes of physiologic saline for five to seven days. Sodium azide was added to give a final concentration of 0.02% to prevent bacterial or fungal growth. Agar gels



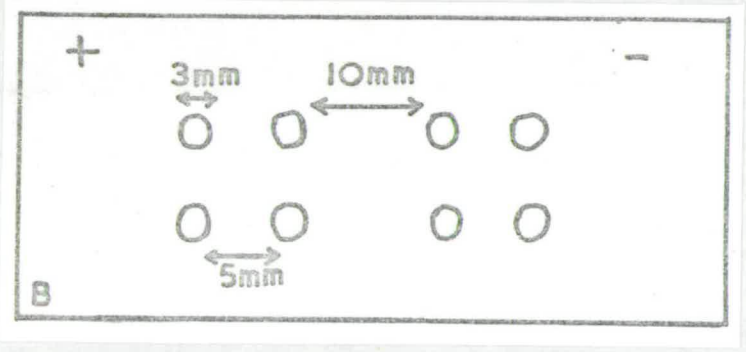


Figure 22:Templates for immunoelectrophoresis(A) and immunoelectroosmophoresis(B)

from the I.E. or I.E.O. tests were placed in daily changes of physiologic saline with 0.02% sodium azide for 72 hours.

ii) Drying of agar gels

Filter paper was placed on the surface of the agar gels to prevent cracking or uneven drying of the gel surface and the gels were left overnight at room temperature. When dry, the filter paper was separated from the gel by soaking in water and any traces of filter paper were removed from the gel by washing.

iii) Staining of agar gels

Dried agar gels were placed in 1% aqueous Ponceau S, a red protein stain, for 20 minutes. Excess stain was removed by successive changes of 6.5% acetic acid. The stained gels were left to dry at room temperature and stored between sheets of filter paper for subsequent assessment.

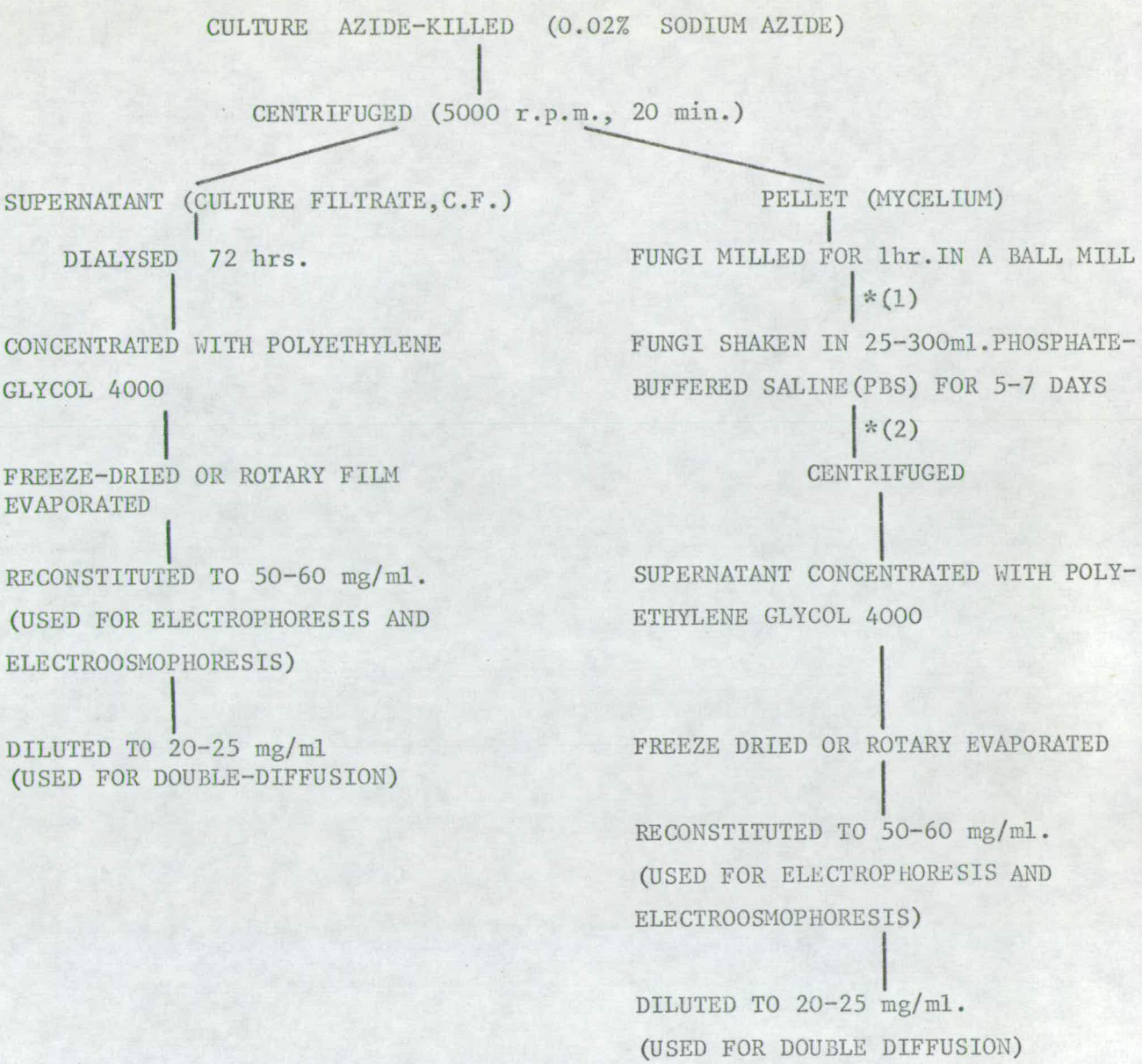
5) Assessment of Precipitin Arcs formed in Serological Tests

In the D.D. test, precipitin arcs which stained to give hard red lines were regarded as strong reactions and those which gave less dense, but still discrete lines, were regarded as weak reactions. Very faint lines and diffusely stained regions were noted but were not included in the results.

In the I.E. test the numbers of precipitin arcs formed were noted and described according to the classification of A, B and C arcs made by Pepys and Jenkins, 1965.

The I.E.O. test was considered positive when one or more hard red lines were seen between the antigen and antiserum wells.

The reactivity of particular antigenic fractions to sera was compared with the microbiology of the hay, grain or dust samples from which the antigens were extracted.



*(1) Actinomycete pellet held in an ice bath and sonically disintegrated at 8μ peak to peak for 5 bursts of 30 secs.

*(2) Actinomycete pellet, after sonication, shaken in 25 ml. PBS for 5-7 days.

FIGURE 21. FLOW CHART OF ANTIGEN EXTRACTION FROM FUNGI AND ACTINOMYCETES.

Results.

Examples of precipitin arcs formed in serological tests by the double-diffusion, imnunoelectrophoresis and immunoelectroosmophoresis techniques are shown in Figures 23 and 24.

A. The reactions of sera to standard farmer's lung hay (F.L.H.) antigen and to antigens from type cultures of Micropolyspora faeni and Thermoactinomyces vulgaris.

a) F.L.D. Patients

In Table 34, it can be seen that, of 11 sera from FLD cases, 3 gave reactions of three to six precipitin arcs to standard F.L.H. in the double-diffusion test. Reactions of one precipitin arc were recorded for 3 more FLD patients but the remaining 5 were negative. Of these 5, four were positive to one test only of the following: fluorescent antibody, I.E. and also D.D. tests involving C.F. and M. extracts of strains of M. faeni from Ayrshire farms. The 3 sera highly reactive to F.L.H. were also strongly positive in all other tests to which they were subjected. Sera from patients B and 23 were in short supply, allowing only limited testing for this section. Sera from only two cases of FLD reacted to the C.F. extracts of T. vulgaris and none reacted to the M extract of this organism. Only one FLD serum, 36, was negative to all tests in this section.

b) Non-FLD personnel

Eight of the 27 sera from non-FLD personnel were positive to FLH antigen, there being 3 with three to six precipitin arcs, 1 with two precipitin arcs and 1 with one precipitin arc from those on FLD farms. Of personnel on non-FLD farms, 1 serum gave two precipitin arcs and 2 gave one precipitin arc.

(i) Non-FLD personnel on non-FLD farms

Three of the sera strongly reactive to FLH also reacted in all other tests except serum 17 which was negative in the I.E.O. test. Serum 6, which gave a two-arc reaction to FLH was positive to F.A.

Figure 23:The reactivity of serum from FLD patient,C (c,d,e,f and g) to culture filtrate extracts of Micropolyspora faeni(g),to F.L.H. antigen(d), and to extracts of hays(c),whole grains(d), bruised grains(e) and dusts(f) and of serum 22 to culture filtrate(C.F.) and mycelial(M) extracts of M.faeni and of colony type 802/5.

a)1.M.faeni,farm strain L.M.(M)
2. " " " B.M.(CF)
3. " " " A (CF)
4. " " " A (M)
5. " " " L.M.(CF)

b)1.802/5 strain L.M. (M)
2.802 " A (CF)
3.802 " W.H. (CF)
4.802/5 " L.M. (CF)

c)1. C52(B.P.)
2. C1 (L.M.)
3. T1 (L.M.)
4.C34 (K.M.)
5.T34 (K.M.)

d)1. T11(M.F.)
2. FLH standard antigen
3.C42(B)
4.T42(B)
5.C11(M.F.)

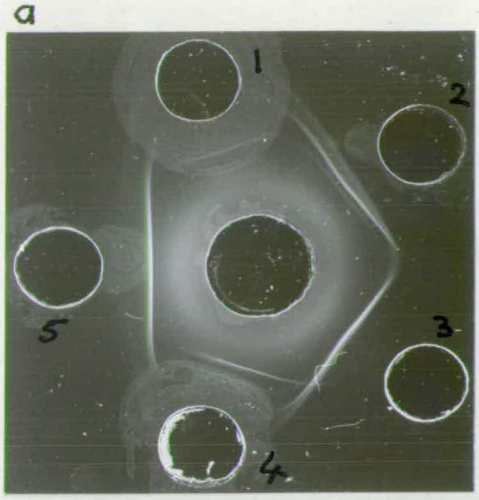
e)1.T15 (B.M.)
2.T5 (S.B.)
3.C41 (A)
4.T41 (A)
5.C15 (B.M.)

f)1.C3 (S.B.)
2.T3 (S.B.)
3.C31(K.M.)
4.T31(K.M.)
5.C13(B.M.)

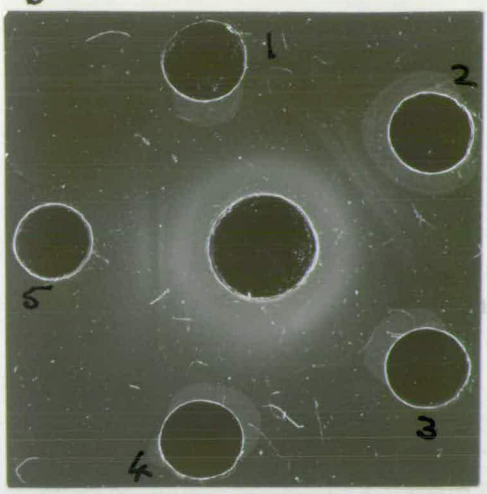
g)M.faeni,strain L.M.
2. " " B.M.
3. " ,Lacey strain
4:M.faeni,strain W.H.
5. " " S.B.
6. " " A.

C=extracted by Coca's solution.
T=extracted by 5% trichloracetic acid.

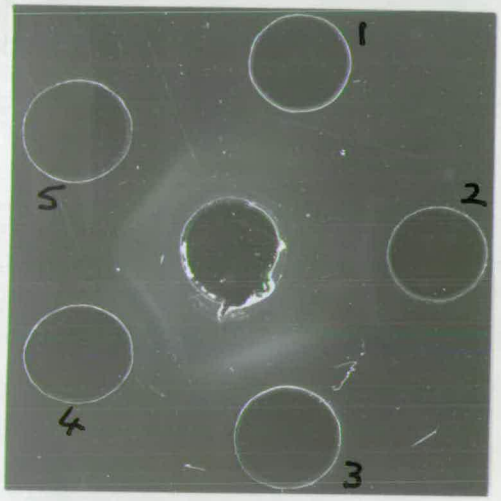
a



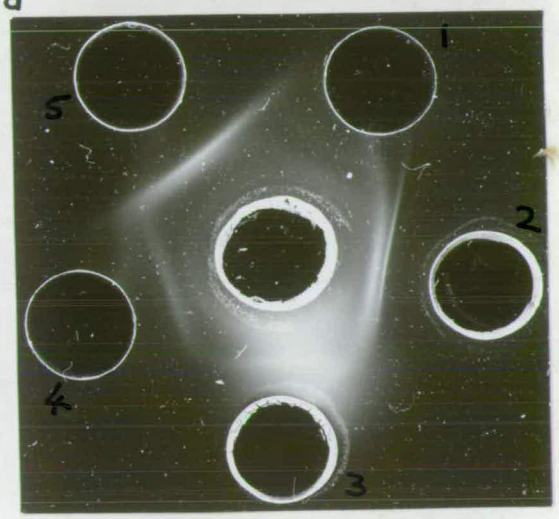
b



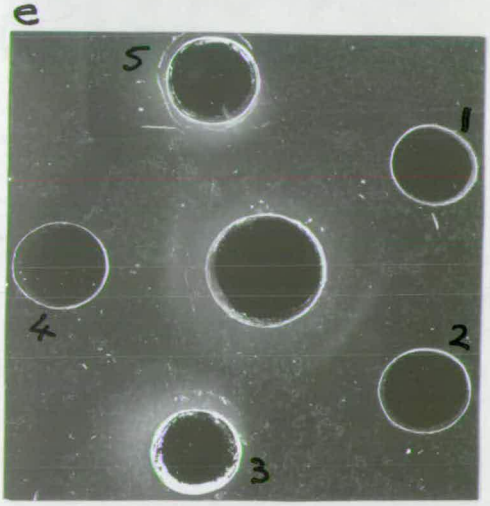
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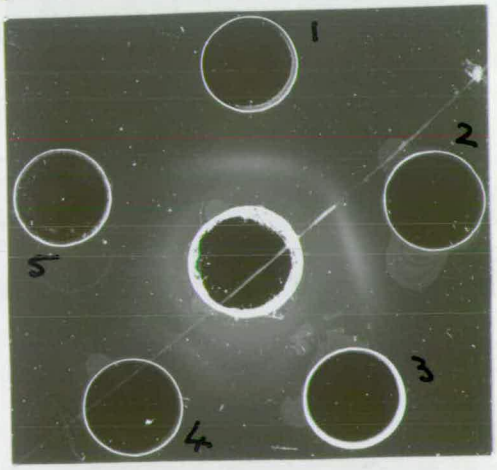
d



e



f



g

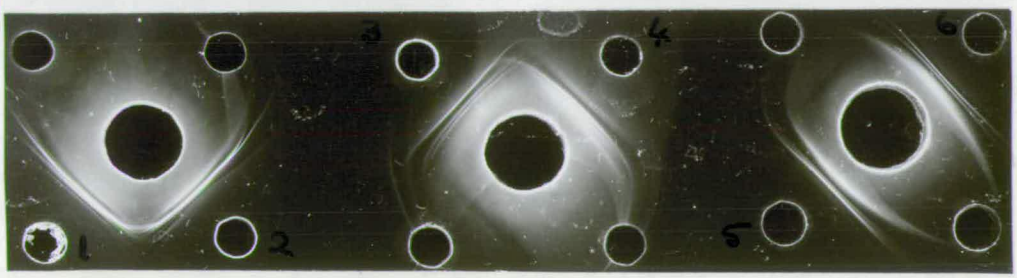


Figure 24: Arcs of precipitation formed when serum of FLD patient, C, was tested against culture filtrate extracts of *Micropolyspora faeni* by immunoelectrophoresis(a-g) and immunoelectroosmophoresis(h).

a) *M.faeni*, Lacey strain.
b) *M.faeni*, farm strain W.H.
c) " " " A.
d) " " " L.M.
e) " " " E.M.
f) " " " S.B.
g) " " " M.H.
h) *M.faeni*, Lacey strain.

All extracts were used at a concentration of 40mg/ml. The A, B and C regions of reactivity in immunoelectrophoresis are indicated.

a



b

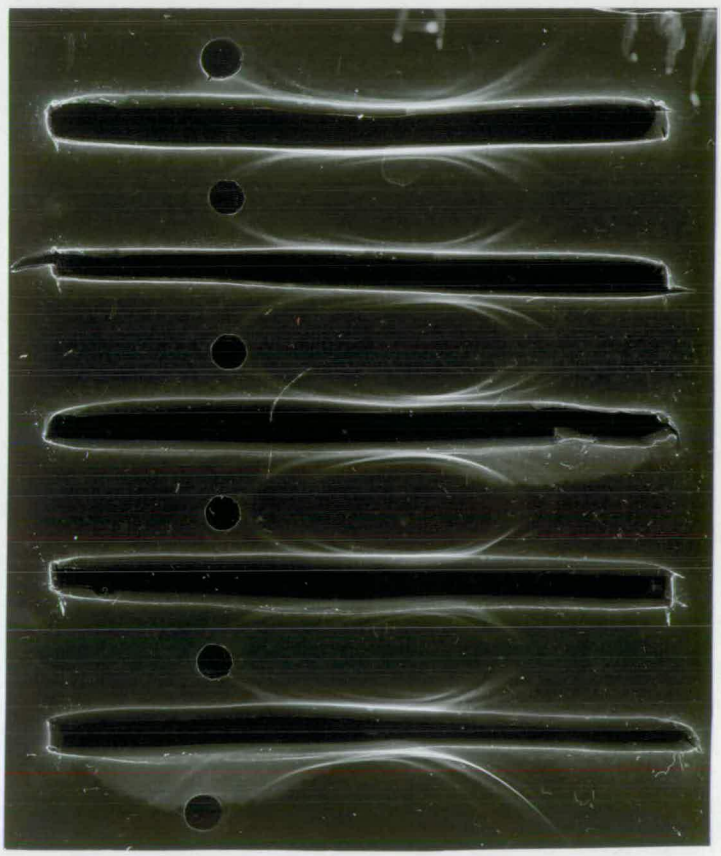
c

d

e

f

g



h

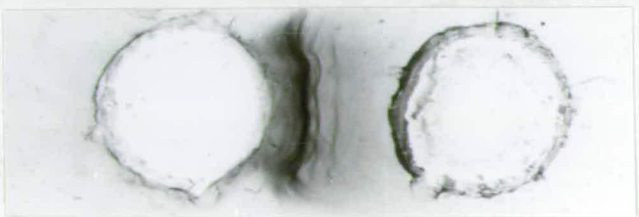


Table 34 : Legend

D.D. : Double diffusion test

I.E. : Immunoelectrophoresis test

I.E.O.: Immunoelectrosmophoresis test

F.L.H.: Standard F.L.H. 6.5mg./ml.

C.F.(L)(i) : Culture filtrate of M. faeni (Lacey strain). 20mg/ml.

C.F.(L)(ii) : " " " " " 40mg/ml.

F.A. : Indirect fluorescent antibody technique.

M. faeni (Ayr) : The culture filtrate (C.F.) and mycelial (M) extracts of 6 strains of M. faeni from Ayrshire farms, 20-25mg/ml.

802/5 (Ayr) : C.F. and M. extracts of 4 strains of M. faeni variants from Ayrshire farms, 20-25mg/ml.

T. vulgaris: C.F. and M extracts of T. vulgaris (Lacey strain) 20-25mg/ml.

(1) : 1 precipitin arc formed.

1/6 : 1 positive reaction to 6 extracts.

A,B,C : Type of precipitin arc, according to Pepys and Jenkins 1965.

N.R. : No result

* : Positive before staining.

Serum Group	Blood No.	D.D. FLH	D.D. C.F. (L)(i)	I.E. C.F. (L)(ii)	I.E.O. C.F. (L)(ii)	F.A.	D.D. M.faeni (Ayr) C.F.	D.D. M.faeni (Ayr) M.	D.D. 802/5 (Ayr) C.F.	D.D. 802/5 (Ayr) M.	D.D. T.vulgaris (L) C.F.	D.D. T.vulgaris (L) M.
FLD	2	+(1)				+	1/6	1/6				
	7	+(1)							2/4			
	10			+ *								
	13						6/6	5/6	1/4			
	15					++						
	21					+						
	23	+(3)	+(2)	+A,B,C	+	++	5/6	N.R.	N.R.	N.R.	N.R.	N.R.
	28	+(1)				+	6/6	5/6	2/4			
	36											
	B	+(4)	+(4)	+A,B,C	+	N.R.	N.R.	N.R.	N.R.	N.R.	+(1)	
	C	+(6)	+(4)	+A,B,C	+	N.R.	6/6	6/6	3/4	2/4	+(1)	
NON-FLD ON FLD FARMS	1	+(1)										
	8	+(2)				+	1/6	2/6	2/4			
	9	+(1)	+(1)	+ *	+	+						
	32					+						
	SERA NUMBERS 11, 12, 16, 27, 29, 30, 31, 33, 34, 35 NEGATIVE IN ALL TESTS											
NON-FLD ON NON-FLD FARMS	3	+(1)				+		1/6	2/4			
	5					+	2/6	2/6	2/4	1/4	+(1)	
	6	+(2)				++		2/6	2/4	1/4	+(1)	
	17	+(3)	+(1)	+A,B		++	2/6	5/6	1/4	2/4	+(1)	
	22	+(6)	+(4)	+A,B,C	+	++	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
	25	+(5)	+(2)	+A,B,C	+	++	6/6	6/6	3/4	2/4	+(2)	
	26					+	N.R.	N.R.	N.R.	N.R.		
	SERA NUMBERS 4, 14, 18, 19, 20, 24 NEGATIVE IN ALL TESTS											

Table 34: The reactions of 38 sera to farmer's lung hay (FLH) antigen and to antigens from Micropolyspora faeni and Thermoactinomyces vulgaris.

and also when tested against strains of M. faeni. A similar result was recorded for sera 3 and 5 but serum 5 was negative to FLH. Four of the sera in this group which were positive to other tests reacted to the C.F. of T. vulgaris. Six of the thirteen sera in this group reacted to none of the antigens in this section.

(ii) Non-FLD personnel on FLD farms

Of the three sera positive to FLH antigen, serum 8 reacted to F.A. and to extracts from strains of M. faeni, showing a pattern of reactivity similar to sera 3 and 6 from non-FLD farms. Serum 9 was positive in all tests except those against strains of M. faeni from Ayrshire farms. Serum 32 reacted weakly in the F.A. test only. No sera were reactive to C.F. or M extracts of T. vulgaris. Ten of the 14 sera in this group were negative in all tests.

Types of serological reactivity

1. There appeared to be four types of reactivity of sera to tests excluding those to extracts of T. vulgaris.

i) To all tests to which subjected: B,C,17(?), 22,23,25 (17 negative to I.E.O.)

ii) To FLH, F.A. and local strains of M. faeni: 2, 7 (?), 28, 8,3, 5 (?), 6. (7 negative to F.A.; (5 negative to F.L.H.)

iii) To all tests except to local strains of M. faeni: 9

iv) To one test only. 10, 13, 15, 21, 1, 32,26.

2. Of six sera reacting to C.F. of T. vulgaris (Lacey strain) four sera had reactivity of type (i) and 2 had reactivity of type (ii) T. vulgaris (L :C.F.) reacted with all those sera having between 2 and 6 precipitin arcs to FLH to which it was tested and therefore showed a strong correlation with reactivity to FLH. The only exception was serum 5 with a positive reaction to T. vulgaris (C.F.) but a negative reaction to FLH antigen. (Mycelial and C.F. extracts of a strain of

T. vulgaris isolated from an Ayrshire farm were also tested against sera but no precipitin arcs were formed).

3. As shown in Table 34 seventeen sera were negative to all tests (1 of the eleven FLD patients, 6 of the thirteen non-FLD personnel on non-FLD farms and 10 of the fourteen non-FLD personnel on FLD farms). There was therefore, a marked lack of reactivity by sera from non-FLD personnel on FLD farms.

4. The distribution of sensitisation was almost equal amongst personnel on FLD farms (52%) and non-FLD farms (54%), omitting sera B and C which had no comparable non-FLD sera. The degree of sensitisation, as indicated by the strength of serological reactions, was also similar. On FLD farms, however, 8/12 (67%) of sensitised individuals were additionally symptomatic.

None of the control sera from the Blood Transfusion Service were positive to standard FLH antigen.

B. The reactions of sera to antigenic extracts of samples of hay, grain and dust from FLD and non-FLD farms.

Of the six highly reactive sera described in 1(i) above, four gave high numbers (between 12 and 28) of strong reactions and one, 25, gave six strong reactions to those of the sixty antigenic extracts of hays, grains and dusts to which they were tested (Tables 35 and 36). Neither serum 23 nor 26 was tested as only small quantities were available. All other sera gave no strong reactions apart from serum 29, with five reactions, serum 5 and serum 6 with three, two sera each with two reactions and eight sera, each with one reaction. These results seem to indicate that sera highly reactive to FLH and to extracts of strains of M. faeni (Table 34) were also highly reactive to a wide range of extracts of natural substrates. (Tables 35 and 36). Of the eight sera which reacted weakly to FLH, three gave no reactions to any of the 60 extracts of farm products tested while the remaining five gave single positive reactions only. Nine sera which were positive when tested against natural

Serum Group	Serum Number	ANTIGENIC EXTRACTS TESTED				HAZARD		
		I		II	III	HAY	GRAIN	
		Strong	Weak				(Harvest)	(After Storage)
FLD	2		7	1	4	++		+
	7		6				++	
	10		5			++	++	++
	13	1	7			++		++
	15		7			++	++	++
	21		5	1				++
	23	N.R.	N.R.	N.R.	N.R.	++		
	28	1	6		1	++		
	36		8			++	++	
	B	25^{x}	4^{x}		4	++		+
	C	21	15	1	1	++		++

Table 35a

Table 35 : The reactions of 38 sera from personnel with (FLD) and without (non-FLD) farmer's lung disease from FLD and non-FLD farms, to the following;

I : 60 antigenic extracts of 30 samples of hay, whole and processed grain and dust (both strong and weak reactions are given)

II : 32 culture filtrate (C.F.) and mycelial (M) extracts of mesophilic and thermophilic fungi.

III : 16 C.F. and M. extracts of thermophilic actinomycetes and bacteria

x : 36 extracts tested.

Table 35a : The hazards connected with onset of symptoms by FLD patients.
Grain (harvest): symptoms associated with handling grain at harvest-time.
Grain (after storage): symptoms associated with processing or feeding grain to cattle.

++ : main hazard

+ : subsidiary hazard.

Serum Group	Serum Number	ANTIGENIC EXTRACTS TESTED				HAY	GRAIN
		I		II	III		
		Strong	Weak				
NON FLD ON FLD FARMS	1	1	5	1		++	B. ++
	8	1	7		1	++	B. ++
	9		4		2	++	B. ++
	11		6	1		++	B. ++
	12		3	1		+	++
	16		7			++	B. ++
	27	1	2		1	+	
	29	5	15	1	1	++	++
	30	1	6	1		+	+
	31		6			++	++
	32		8	1		++	++
	33		4		1	++	++
	34	2	6			++	++
	35	F	3[F]			+	+
NON-FLD ON NON-FLD FARMS	3	1	13	2	2	++	B. ++
	4		9	1	1	++	B. ++
	5	3	8	1	1	++	++
	6	2	10	1	2	++	++
	14	2	2	1		++	B. ++
	17	12	5		1	++	B. ++
	18	1	7			++	++
	19	2	5	1		++	B. ++
	20		2			++	++
	22	28*	2[*]			++	B. ++
	24		2			++	B. ++
	25	6	1		1	++	++
	26	N.R.	N.R.			+	

Table 35b: Legend as for Table 35, but

F	:	50 extracts tests
*	:	38 extracts tested
Hay	:	Personnel in contact with hay
Grain	:	Personnel in contact with grain
++	:	frequent contact
+	:	occasional contact
B	:	contact during bruising or milling of grain.

Serum Group	Serum No.	HAY SAMPLES(9) FLD(4) C	T	Non-FLD(5) C	T	WHOLE GRAIN SAMPLES(8) FLD(5) C	T	Non-FLD(3) C	T	PROCESSED GRAIN SAMPLES (5) FLD(3) C	T	Non-FLD(2) C	T	DUST SAMPLES(8) FLD(5) C	T	Non-FLD(3) C	T
FLD	13							1									
	23	N.R.		N.R.		N.R.		N.R.		N.R.		N.R.		N.R.		N.R.	
	28					1											
	B	1/3	2/3	3/3	2/3	2/3	3/3	3	2	N.R.		N.R.		1/3	2/3	2	2
	C	1	2		1	4	1			3	1	2		2	1	2	1
SERA NOS. 2,7,10,15,21,36 : NO 'STRONG' REACTIONS																	
NON FLD ON FLD FARMS	1					1											
	8	1															
	27					1											
	29		1	1			1							1	1		
	30		1														
	34		1					1									
SERA NOS. 9,11,12,16,31,32,33,35 : NO 'STRONG' REACTIONS																	
NOM-FLD ON NON; FLD FARMS	3	1															
	5	1				2											
	6	2															
	14		1				1										
	17	2	3		1	1	1	1						2	1		
	18		1														
	19		1				1										
	22	3/3	2/3	2	1	3/3	3/3	2	2	N.R.		N.R.		3/3	3/3	2/2	2/2
	25	2	1			1		1	1								
	26	N.R.		N.R.		N.R.		N.R.		N.R.		N.R.		N.R.		N.R.	
SERA NOS. 4,20,24 : NO 'STRONG' REACTIONS																	
TOTAL NO. OF REACTIONS OF SERA		14	16	6	5	16	11	9	5	3	1	2	0	9	8	6	5

Table 36: The 'strong' reactions of 38 sera from personnel with (FLD) and without (non-FLD) farmer's lung disease from FLD and non-FLD farms to antigenic extracts of samples of hay, whole and processed grain and dust from these farms.

C : antigenic fraction extracted by Coca's solution

T : antigenic fraction extracted by 5% trichloracetic acid

( ) : The number of samples in each group

N.R. : No result.

substrates were negative to FLH.

Weak reactions, of between one and fifteen per serum, to the 60 antigenic extracts of natural substrates were recorded for all sera (Table 35).

The antigenicity of samples of hay, grain and dust from FLD and non-FLD farms.

a) in relation to the sources of samples

The number of 'strong' reactions of antigenic extracts of hays, whole and processed grains and dusts to all sera except numbers 23 and 26, are shown in Table 36. The four hays which were all 'bad' hay samples were more reactive. Extracts from the four 'bad' hay samples from FLD farms were more reactive than those from non-FLD farms. This also applied to samples of whole and processed grain and to samples of dust. The hay samples from FLD farms yielded the most reactive extracts, closely followed by whole grain samples from FLD farms. Dusts from FLD farms were similar in reactivity to whole grain samples from non-FLD farms and hay and dust samples from non-FLD farms realised similar, slightly lower values. Processed grain samples gave very low values but this was probably due to the fact that the highly reactive sera, B and 22, were not tested against extracts of these materials. The fact that serum C reacted strongly to extracts from samples from both FLD and non-FLD farms seems to indicate that processed grain samples contain antigens capable of reacting with a serum which was highly reactive both to F.L.H. and to extracts of M. faeni (Table 34).

The majority of reactions were from the four sera, B, C and numbers 17 and 22. Sera B and 22 demonstrated a particular lack of specificity in their strong reactions to all types of extracts to which they were tested. Sera C, 17 and 25 reacted more strongly with samples from FLD farms than those from non-FLD farms but it was the few, less common reactions of several other sera from non-FLD personnel which caused the

final total to be significantly higher for samples from FLD farm products. It may have been significant that only the highly reactive serum, C, reacted with extracts of processed grain and with dust from non-FLD farms. All other types of product had at least three strong reactions to sera outwith the highly reactive group of sera (Table 36). It therefore seems that sensitisation to antigens extracted from all types of samples from FLD farms, excluding those of processed grain, was higher in a wide range of sera from non-FLD personnel on both FLD and non-FLD farms.

b) in relation to the method of antigen extraction from samples

There were more positive reactions to extracts of the same materials obtained by using Coca's solution (C) than to those obtained by using 5% T.C.A., apart from those of hay samples from FLD farms (Table 36). This did not apply to individual samples and in many cases T.C.A.extracts were more reactive than C extracts, as in the case of whole barley sample E.M.14 (Table 38).

c) in relation to the microbiology of samples

Part of the microbiology of the samples used in serological testing, summarised from the findings in Section III of this thesis, are shown in Tables 37, 38 and 39 with the total numbers of weak and strong reactions of sera to C and T.C.A. extracts of these samples.

The number of sera reactive to samples was not related to the numbers of mesophilic organisms isolated from all types of sample examined. In the case of hays (Table 37), reactivity may have been loosely associated with the mesophilic groups or species isolated from the 'bad' hay samples K.M.34, B.P.52 and M.H. 36 which were different from those isolated from other hay samples.

With one exception (hay sample M.F.10), there was a correlation between the total number of 'strong' reactions of sera to hay samples and the numbers of thermophilic actinomycetes and bacteria isolated from these samples (Table 37). Of four 'bad' hay samples from FLD farms,

Hay Sample	Good (G) or Bad (B)	FLD(+) or Non-FLD (-)	Total M26°C	Dominant Groups or Species M26°C	No. Cols per 10g. N+C 46°C	No. Cols M.faeni per 10g N+C 46°C	Number of Reactions of Antigenic Extracts of Samples to Sera							
							COCA			T.C.A.			Total	
							St.	Wk.	Tot.	St.	Wk.	Tot.	St.	Wk.
L.M.1	G(?)	+	71	A.glaucus Trichoderma sp.	2,959	2,862	3	24	27	4	13	17	7	37
K.M.34	B	+	3634	Yeasts Actinomycetes	2,550	2,450	4	4	8	2	10	12	6	14
B.P.52	B	+	17947	Yeasts A. fumigatus Mucorales Actinomycetes	12,298	6,143	1	9	10	7	12	19	8	21
M.H.36	B	+	4036	A.versicolor A.nidulans Penicillium spp	1,107	14	6	1	7	3	1	4	9	2
M.F.10	G	-	229	A.glaucus	6	0	2	3	5	4	2	6	6	5
W.H.18	G	-	45	A.glaucus	10	6	1	1	2	1	13	14	2	14
B.38	G	-	1602	A.restrictus Actinomycetes	10	1	2	0	2	0	1	1	2	1
M.T.17	G	-	911	A.restrictus A.versicolor	95	69	1	4	5	0	1	1	1	5
B.M.27	G	-	3715	A.versicolor	200	38	0	0	0	0	1	1	0	1

Table 37: The number of colonies and dominant groups of species of mesophilic organisms (M26°C); the numbers of colonies of thermophilic actinomycetes and bacteria (N+C 46°C) and M. faeni isolated from good and bad hay samples from farmer's lung disease (FLD) and non-FLD farms. The strong (St.), weak (Wk.) and total (Tot.) serologial reactions of antigenic extracts of these samples to 36 sera from personnel on FLD and non-FLD farms.
Coca: antigenic fraction extracted by Coca's solution.
T.C.A.: antigenic fraction extracted by 5% trichloracetic acid.

Whole Barley Sample --	FLD (+) or Non-FLD (-)	Total M26°C	Dominant Groups or Species M26°C	No. Cols per 10g N+C46°C	No. Cols M.faeni per 1og. N+C46°C.	Number of Reactions of Antigenic Extracts of Samples to Sera							
						COCA			T.C.A.			TOTAL	
						St.	Wk.	Tot.	St.	Wk.	Tot.	St.	Wk.
S.B.6	+	5359	Penicillium spp	802	618	6	5	11	2	4	6	8	9
K.M.32	+	2556	A.candidus Actinomycetes	2334	1821	7	10	17	2	0	2	9	10
E.M.14	+	7076	Penicillium spp Yeasts	271	1	1	3	4	6	2	8	7	5
A.43	+	28	Yeasts	5	1	1	0	1	1	0	1	2	0
B.P.48 (Oats)	+	509	Penicillium spp Yeasts	179	13	1	0	1	0	0	0	1	0
W.H.19	-	5	Yeasts	125	60	5	0	5	2	0	2	7	0
B.42	-	2273	Penicillium spp Geotrichum sp Yeasts	160	46	1	0	1	2	0	2	3	0
M.F.11	-	5796	Penicillium spp Yeasts	8	0	3	0	3	1	0	1	4	0

Table 38: The numbers of colonies and dominant groups or species of mesophilic organisms (M26°C); the numbers of colonies of thermophilic actinomycetes and bacteria (N+C 46°C) and M. faeni isolated from whole barley samples from farmer's lung disease (FLD) and non-FLD farms. The strong (St.), weak (Wk.) and total (Tot.) serological reactions of antigenic extracts of these samples to 36 sera from personnel on FLD and non-FLD farms.

Coca: antigenic fraction extracted by Coca's solution.
T.C.A.: antigenic fraction extracted by 5% trechloracetic acid.

each with a high count for numbers of thermophilic actinomycetes and bacteria, three released high numbers of propagules of M. faeni/10g. Sample M.F.10 from a non-FLD farm released very few thermophilic actinomycetes and bacteria but gave six strong reactions with sera, more than any other hay sample from a non-FLD farm. The distribution of weak reactions, in general, correlated with that of the strong reactions. One exception was the high number of weak reactions to the T.C.A. extract of the hay sample, W.H.18, from a non-FLD farm.

Of three whole barley samples from FLD farms with high serological reactivity in the form of both strong and weak reactions (Table 38), two (S.B.6 and K.M.32) yielded large numbers of thermophilic actinomycetes and bacteria, predominantly of M. faeni. The third sample, E.M.14, released relatively low numbers of colonies of thermophilic actinomycetes and bacteria but was strongly reaction to seven sera, and weakly reactive to five. No weak reactions were recorded from sera tested against the three non-FLD samples but the numbers of strong reactions were relatively high. Two of the three samples from non-FLD farms yielded appreciable numbers of colonies of thermophilic actinomycetes and bacteria, including M. faeni.

There was no correlation between the numbers of colonies of thermophilic actinomycetes and bacteria from processed grain and the low numbers of strong reactions to such samples (Table 39).

A situation almost identical to that for whole barley samples existed for dust samples (Table 39). Three FLD farms contained dust samples which, when extracted, reacted strongly with sera. Two of these dusts, S.B.3 and K.M. 31 were the only samples of this type to release appreciable numbers of propagules of M. faeni while the third sample, E.M.13, released none. Three dust samples from the non-FLD farms with whole barley samples which reacted strongly to sera, also reacted strongly. A larger number of weak reactions to extracts of dust was recorded than to extracts of whole or processed grain samples from both FLD and

Processed Barley Sample	FLD (+) or Non-FLD (-)	Total M26°C	Dominant Groups or Species M26°C	No. Cols per 10g. N+C46°C	No. Cols *M. faeni* N+C46°C	Number of Reactions of Antigenic Extracts of Samples to Sera							
						COCA			T.C.A.			TOTAL	
						St.	Wk.	Tot.	St.	Wk.	Tot.	St.	Wk.
S.B.5	+	11781	Penicillium spp.	1576	1182*	1	0	1	0	0	0	1	0
A.41	+	2250	Bacteria	132	40	1	0	1	1	0	1	2	0
E.M.15	+	21	Yeasts Geotrichum sp	3	0	1	11	12	0	0	0	1	11
M.F.16	-	3384	Penicillium spp Geotrichum spp Paecilomyces spp	1	0	1	0	0	0	0	0	1	0
B.40	-	4355	Geotrichum spp Bacteria	21	0	1	0	0	0	0	0	1	0
Dust Sample													
S.B.3	+	1689	Yeasts	430	73	4	11	15	2	0	2	6	11
E.M.13	+	2	Penicillium spp	239	0	2	8	10	1	11	12	3	19
K.M.31	+	54	Actinomycetes	46	28	3	7	10	5	0	5	8	7
A.24	+	28	Penicillium spp	20	1	0	9	9	0	0	0	0	9
B.P.46	+	2	Penicillium spp	6	0	0	2	2	0	2	2	0	4
M.F.12	-	16	Geotrichum sp.	26	0	2	0	2	2	0	2	4	0
W.H.20	-	874	Penicillium spp Yeasts	2	1	2	12	14	1	5	6	3	17
B.37	-	6713	Yeasts Penicillium spp. Geotrichum spp	27	4	2	15	17	2	2	4	4	17

Table 39: The number of colonies and dominant groups or species of mesophilic organisms (M26°C); the numbers of colonies of thermophilic actinomycetes and bacteria (N+C46°C) and of *M. faeni* isolated from processed barley and dust samples from farmer's lung disease (FLD) and non-FLD farms. The strong (St.), weak (Wk.) and total (Tot.) serological reactions of antigenic extracts of these samples to 36 sera from personnel on FLD and non-FLD farms.

Coca: antigenic fraction extracted by Coca's solution.
T.C.A.: antigenic fraction extracted by 5% T.C.A.
* : *M. faeni* variant?

non-FLD farms.

C. The reactions of sera to extracts of mesophilic, thermophilic and thermotolerant fungi and to extracts of thermophilic actinomycetes and bacteria.

i) Mesophilic and thermophilic fungi

Table 40 shows the positive reactions of thirty-six sera (excluding 23 and 26) tested against culture filtrate (C.F.) and mycelial (M) extracts of the following mesophilic fungi; Aspergillus candidus, A.fumigatus, A. glaucus (2 strains), A. nidulans, A. restrictus, A.terreus, A.versicolor, Cladosporium sp. Geotrichum sp.,Penicillium sp. and Rhizopus sp., Four of the twelve C.F. and four of the twelve M extracts reacted with at least one of the sera tested. Only one extract reacted with more than one serum (the C.F. extract of the Penicillium sp. reacted with ten sera). Only seven positive reactions resulted when the sera were tested against C.F. and M. extracts of two strains of the thermotolerant A. fumigatus and to extracts of a thermophilic Humicola sp. and a Mucor sp. One reaction was to a C.F. extract of A. fumigatus and the remainder were to M. extracts of the four thermotolerant or thermophilic fungi.

ii) Thermophilic actinomycetes and bacteria

Of the sixteen C.F. and M extracts of thermophilic actinomycetes and bacteria, only two gave reactions with any of the thirty-seven sera tested. (Serum 23 was not tested). A C.F. extract of a white Streptomyces sp.reacted with fifteen sera, distributed throughout the three main serum groups (Table 40). Only serum 3 reacted with a C.F. extract of culture number 309, a brown Streptomyces sp. The extracts which did not react to any sera were from cultures of one other white and two other brown Streptomyces spp.,from the blue/grey/green thermophilic actinomycete (page 115) and from two bacteria.

D. Serological reactivity of personnel in relation to their contact with hay, grain and dust.

		MESOPHILIC FUNGI								THERMOPHILIC FUNGI					ACTINOMY-CETES	
Serum Group	Serum No.	Rhiz. C.F.	Clad. C.F.	Pen. M	Asp.f. (i) C.F.	Asp.t. M.	Asp.n. M.	Asp.r. M.	Asp.v. C.F.	Asp.f. (ii) C.F.	Asp.f. (ii) M.	Asp.f. (iii) M.	Hum. M.	Mucor M.	103 C.F.	309 C.F.
FLD	2					+		+				+		+	+	
	21														+	
	28			+												
	B			+			+					+	+			
	C			+											+	
NON-FLD ON FLD FARMS	1														+	
	8			+												
	9	+	+													
	11														+	
	12														+	
	27			+												
	29			+											+	
	30														+	
	32														+	
	33				+											
NON-FLD ON FLD FARMS	3										+			+	+	+
	4									+					+	
	5			+											+	
	6			+					+						+	
	14														+	
	17			+												
	19															
	25			+												

Table 40: The positive reactions of 36 sera tested against culture filtrate (C.F.) and mycelial (M) extracts of mesophilic fungi, thermophilic and thermotolerant fungi and thermophilic actinomycetes and bacteria.

Rhiz: Rhizopus sp.
Clad: Cladosporium sp.
Pen.: Penicillium Sp.
Asp.f: Aspergillus fumigatus
Asp.t: Aspergillus terreus
Asp.n: Aspergillus nidulans
Asp.r: Aspergillus restrictus
Asp.v: Aspergillus versicolor
Hum: Humicola sp.
Mucor: Mucor sp.
103: White thermophilic actinomycete
309: Brown thermophilic actinomyete
(i): Mesophilic grown at 26°C.
(ii): Thermotolerant grown at 46°C-strain 1
(iii): Thermotolerant grown at 46°C-strain 2
FLD: 'Farmer's lung disease'

In Table 35 the results of serological reactivity of personnel to antigenic extracts of samples of hay, grain and dust and of liquid cultures of mesophilic fungi and thermophilic fungi, bacteria and actinomycetes (excluding M. faeni and T. vulgaris) are summarised. There was no correlation between serological reactivity to extracts of samples of hay, grain and dust and that to extracts of either mesophilic or thermophilic micro-organisms.

Hazards implicated by FLD patients in causing their disease.

The main hazards implicated by FLD patients in causing the disease are also included in Table 35a. All FLD patients worked with both hay and grain. Hay, alone, was considered to be the main hazard by four patients, hay and grain together, by five and grain, alone, by the remaining two. Four implicated either shovelling or bagging of grain in bulk at harvest-time in causing symptoms and five implicated the activities of bruising or milling stored grain. There was no correlation between the main alleged hazard or hazards and serological reactivity of individuals to the antigens specified in Table 35 or to those of M. faeni and T. vulgaris, detailed in Table 34.

Products with which non-FLD personnel were in contact.

In Table 35b, the products with which non-FLD personnel were frequently or rarely in contact are shown. Of fourteen non-FLD personnel on FLD farms, three were rarely in contact with hay or grain, ten were regularly in contact with hay, five were regularly involved in bruising or milling grain and six were only regularly in contact with grain when feeding this commodity to cattle.

Of the twelve personnel on non-FLD farms whose sera were tested, all were regularly in contact with hay and four were regularly involved in bruising or milling grain. Three more occasionally bruised or milled grain while four were only in contact with grain while feeding animals. There was no correlation between the degree of serological reactivity of non-FLD personnel and their contact with hay or grain.

Conclusions

1. There were four types of reactivity of the 38 sera from the FLD and non-FLD personnel tested to the following - F.L.H. antigen by double-diffusion; extracts of liquid cultures of the Lacey strain of M. faeni by double diffusion, I.E. and I.E.O.; extracts of liquid cultures of local strains of M. faeni by D.D.; whole cells of M. faeni by the indirect fluorescent antibody (F.A.) technique. These four types of reactivity were;

i) reactive to all tests to which subjected; 6 sera

ii) reactive to F.L.H., F.A. and local strains of M. faeni; 7 sera

iii) Weakly reactive to all tests except to local strains of M. faeni; 1 serum

iv) Reactive to one test only ; 7 sera

Three sera in group (i) were from FLD patients and three were from personnel on non-FLD farms. The distribution of the other types of reactivity was divided, although not equally between sera from FLD and non-FLD farms. Sensitisation of individuals as indicated by reactivity in any of the tests described above was present in 52% of personnel from FLD farms and from 54% of personnel from non-FLD farms. From the former, 67% of sensitised individuals were also symptomatic.

2. The type strain (Lacey) of M. faeni appears to have been as effective as F.L.H. in selecting highly reactive sera but was not as effective in detecting less reactive sera which were less strongly positive to F.L.H. and/or positive to C.F. and M. extracts of local strains of M. faeni.

3. Seventeen sera were negative to all tests in (1) above. The group with the largest proportion of negative serology was that of non-FLD personnel on FLD farms from which ten of the fourteen sera were negative. The serum from only one FLD patient and six of the thirteen sera from non-FLD farms were negative, indicating a correlation between sensitisation and disease in personnel from FLD farms, but not in those from non-FLD

farms.

4. The reactivity of a C.F. extract of the Lacey type culture of T. vulgaris to six sera, correlated with high reactivity of all but one of those sera to F.L.H. perhaps indicative of common antigens in the two extracts tested. (Extracts of a local strain of T. vulgaris did not react with any serum).

5. There was a correlation between high reactivity of sera to F.L.H. and extracts of M. faeni and strong reactivity to extracts of hays, grains and dusts from FLD and non-FLD farms. Less strong reactivity of sera to F.L.H. and extracts of M. faeni did not, however, correlate with an equivalent degree of apparent sensitisation to antigens extractable from hays, grains and dusts. All sera were weakly reactive to between one and fifteen of the sixty extracts of hay, grain or dust and these reactions which were randomly distributed throughout the three main groups of sera, did not correlate with serological positivity to other antigenic fractions.

6. Antigenic extracts of samples of 'bad' hays and whole grain from FLD farms were those with the highest reactivity to sera. On the whole, samples of hays, grains and dusts from FLD farms yielded a higher reactivity than their counterparts from non-FLD farms. (In the case of hay samples, this difference may have been exaggerated by the fact that 'bad' hay samples from FLD farms were used for antigenic extraction). The differential in apparent antigenicity was not so much due to the results obtained from highly reactive sera, of which two were non-specific in their reactivity, but to an apparently random sensitisation of non-FLD personnel to antigens more commonly extracted from samples from FLD farms.

7. There were differences in the antigenicity of extracts of individual samples of hay, grain and dust obtained by Coca's solution and 5% T.C.A., but those from Coca's solution, proved to be generally more reactive.

8. Antigencity of samples was not related to the numbers of mesophilic

organisms isolated from them but may have been, in the case of 'bad' hay samples loosely connected with the mesophilic groups or species isolated. Strong antigenicity of five hay samples correlated with the numbers of thermophilic actinomycetes and bacteria isolated per unit weight, with one exception. Three of these five hay samples also released large numbers of colonies of M. faeni, linking this organism with the antigencity of hay samples which caused strong precipitin arcs to be formed in tests against sera.

9. The low reactivity of extracts of samples of processed grain was probably, in part, due to the fact that these were not tested against two highly reactive sera. The strongly positive reactions of extracts of processed grain to the one reactive serum against which they were tested emphasised the lack of specificity of this group of sera in forming precipitin arcs against extracts of farm products.

10. For samples of both whole grain and dust, the pattern of reactivity was similar in that a relatively high number of strong reactions was recorded for each of the sample types from three FLD farms and also those from three non-FLD farms. In only two sets of samples, both from FLD farms, was the reactivity associated with relatively high numbers of colonies of thermophilic actinomycetes and bacteria or of M. faeni.

11. Those hay samples which reacted strongly with an appreciable number of sera generally reacted weakly with a relatively large number of other sera. In the cases of grain and dust samples, however, strong reactivity to some sera was not associated with weak reactivity to other sera. The large number of weak reactions to extracts of dust samples was probably largely responsible for the random distribution of weak reactions described in 5, above.

12. There was negligible reactivity of sera to extracts of mesophilic and thermophilic fungi and to those of thermophilic bacteria and actinomycetes, excluding M. faeni and T. vulgaris.

13. According to FLD patients, contact with dust from hay was considered to be a marginally more common cause of symptoms than contact with dust from grain either at times of bulk grain movement at harvest or processing of stored grain. Non-FLD personnel were as frequently in contact with both hay and grain as were FLD patients. There was no correlation between reactivity of sera to antigenic fractions of hay, grain, dust or micro-organisms and the products with which personel were in contact or, as in the case of FLD patients, with those products which they considered to have been the main cause or causes of disease symptoms.

SECTION V

ELECTRON MICROSCOPY OF SELECTED SPECIES OF ACTINOMYCETES ISOLATED FROM FARM BUILDINGS OR PRODUCTS.

Electron Microscopy of selected species of actinomycetes isolated from farm buildings or products

Materials and Methods

Two type cultures of *Micropolyspora faeni* and isolates of this and other thermophilic actinomycetes from farm buildings or products were examined by electron microscopy. Cultures were inoculated onto half-strength nutrient agar containing cycloheximide (Appendix A) and grown at 46°C for 4 to 5 days. Several drops of 1.0% buffered osmium tetroxide (Appendix C) were placed on the lid of the inverted Petri-dish containing each culture. This treatment both killed and fixed the material for subsequent microscopic examination. After 10 to 15 minutes, fresh lids were placed on the cultures. Portions of the organisms concerned were transferred to carbon-coated copper grids by gently touching the surface of the colonies with these grids which were, in turn, placed in an AE1, EM6 electron microscope, examined and photographed.

Results

a) *M. faeni*

Electron micrographs of *M. faeni* (Lacey strain A 94), and of the strain obtained from Dr. Christine Dawson, Glasgow are shown in Plates 1 and 2 together with examples of strains from the farm environments surveyed. All colonies of this organism had the characteristics described by Cross et al., 1968 and Cross and Goodfellow, 1973. Colonies were raised, yellow orange to yellow brown with white aerial mycelium. Sporulation in straight chains of up to 5 spores was noted (Plate 1b and c).

b) Monosporic species

Spores and lysed hyphae of *Thermoactinomyces vulgaris* are shown in Plate 3a. This thermophilic actinomycete was fast-growing with dense white aerial mycelium and was resistant to 25 ug/ml. novobiocin (Cross, 1968; Cross and Goodfellow, 1973). The endospores formed by this species are heat-resistant and can survive in soil for considerable lengths of time.

Plate 3 also contains electron micrographs of Saccharomonospora viridis or Thermomonospora viridis (Cross and Goodfellow, 1973), isolated from two different farms. The single spores borne on sporophores arising from the aerial mycelium can be seen. Colonies of this organism were initially white, becoming blue/grey/green within 3 days at 46°C.

c) Streptomyces species

Spores and hyphal fragments of white Streptomyces spp. are shown in Plates 4 and 5 and of brown Streptomyces spp. in Plates 6 and 7. Examples of straight and spiral chains of spores can be seen in Plates 6a and 4a respectively.

d) Bacillus species

To indicate the similar dimensions of actinomycetes and bacteria, an electron micrograph of a thermophilic Bacillus is shown in Plate 7c. This organism was provisionally identified as Bacillus cereus var. mycoides

Plate 1:Electron micrographs of Micropolyspora faeni(201)

a)Mycelium X 4,500

b)Mycelium and spore chain X 6,000

c)Spore chains X 23,450

a



b



c

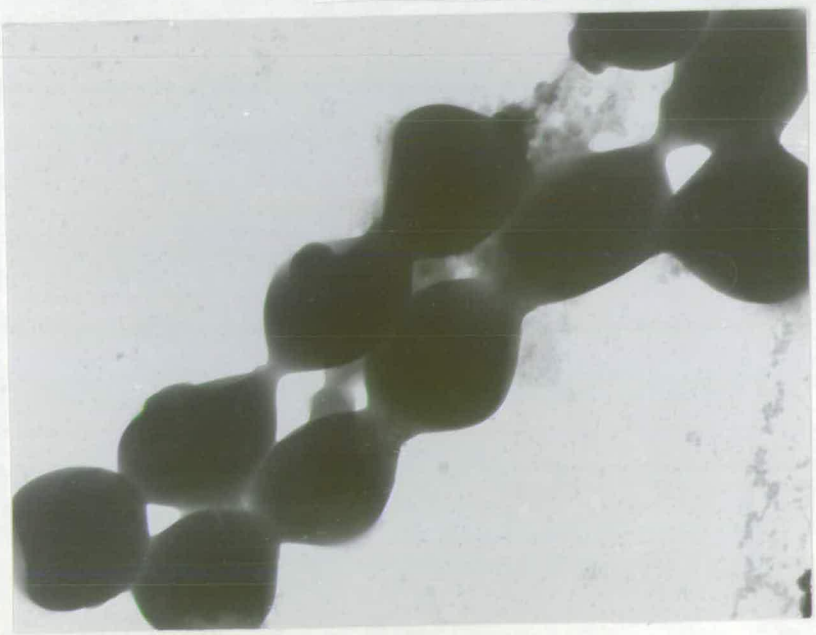


Plate 2:Electron micrographs of strains of
Micropolyspora faeni

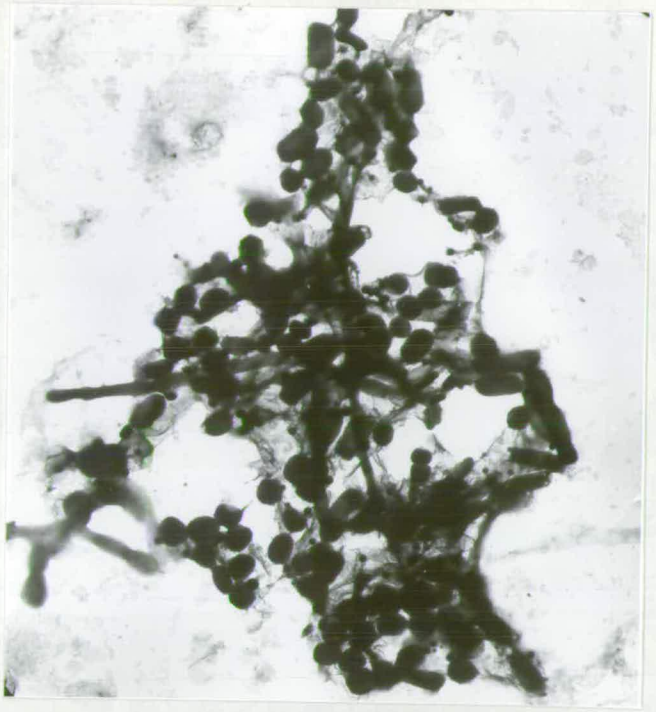
a)Glasgow strain;mycelium and spores X3,600

b)Lacey strain;spore chains X 3,600

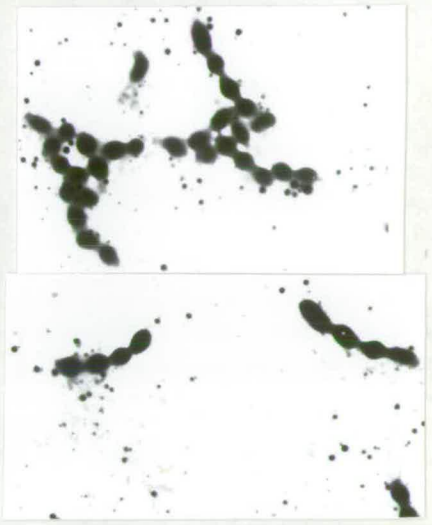
c)Farm strain K.M.203;spore chains X 3,600

d)Farm strain K.M.802;spore chains X 3,600

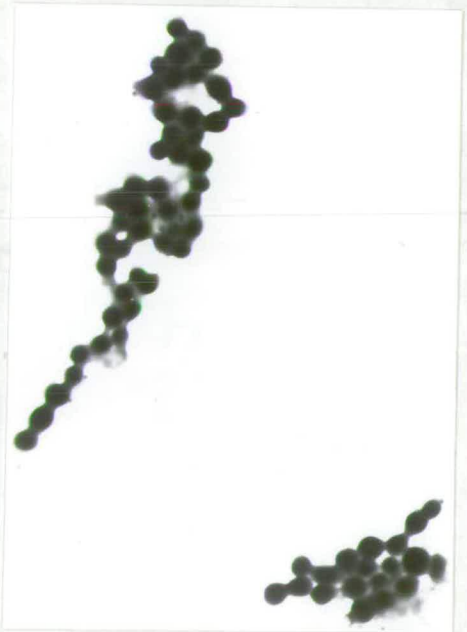
a



b



c



d

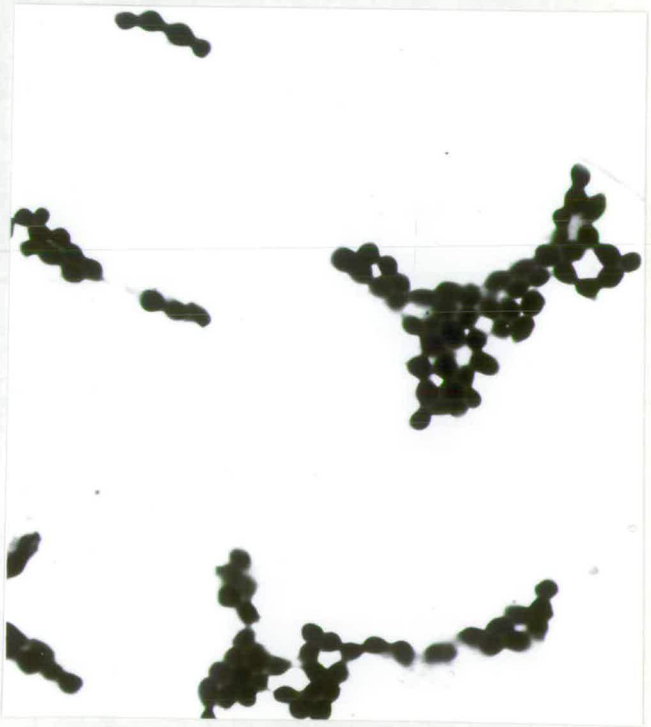


Plate 3:Electron micrographs of thermophilic actinomycetes

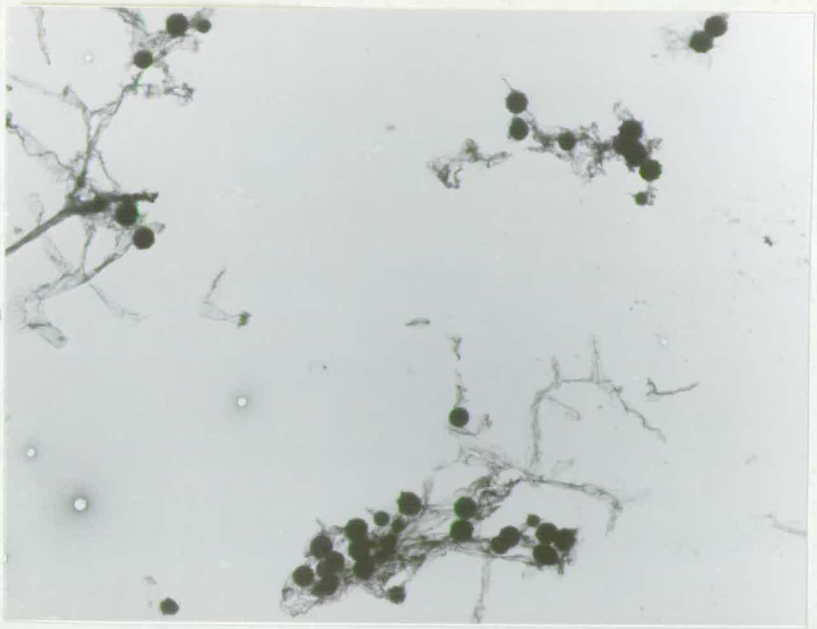
a)Thermoactinomyces vulgaris;lysed hyphae and spores X 4,200

b)Saccharomonospora viridis(Thermomonospora viridis; hyphae and spores X 3,600

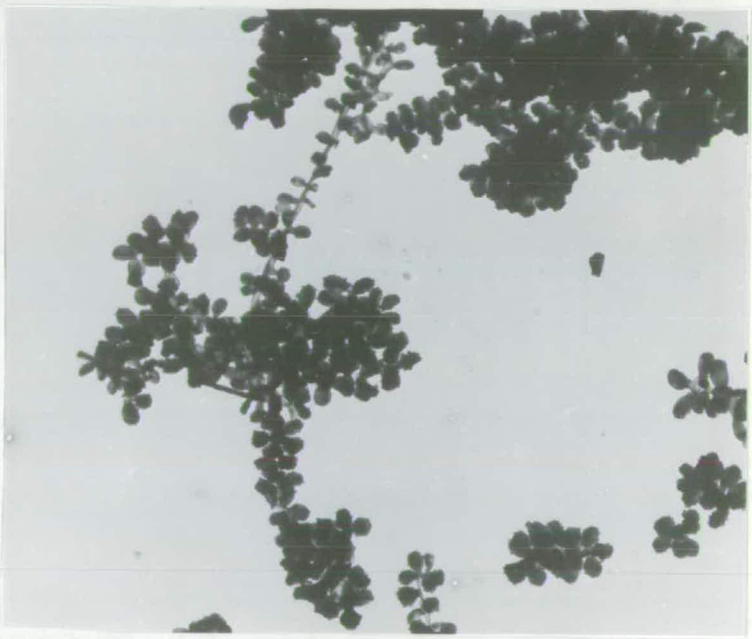
i)Farm strain E.C.

ii)Farm strain L.M.

a



b



c

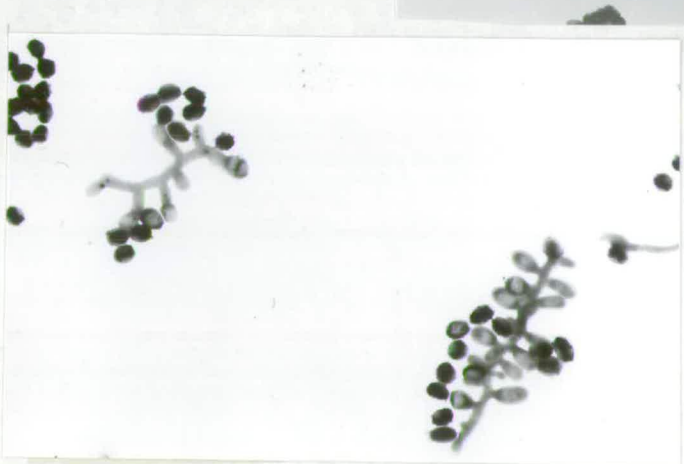


Plate 4:Electron micrographs of white Streptomyces species

a)S.B.103 i)Spiral chains of spores

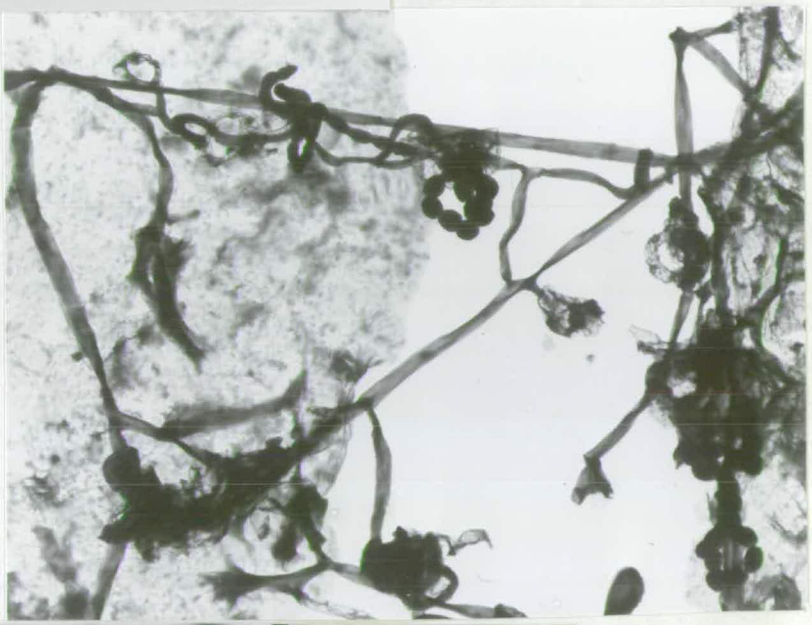
ii)Hyphae and spores X 4,200

b)103;hyphae and spore chains X 7,000

a i)



a ii)



b

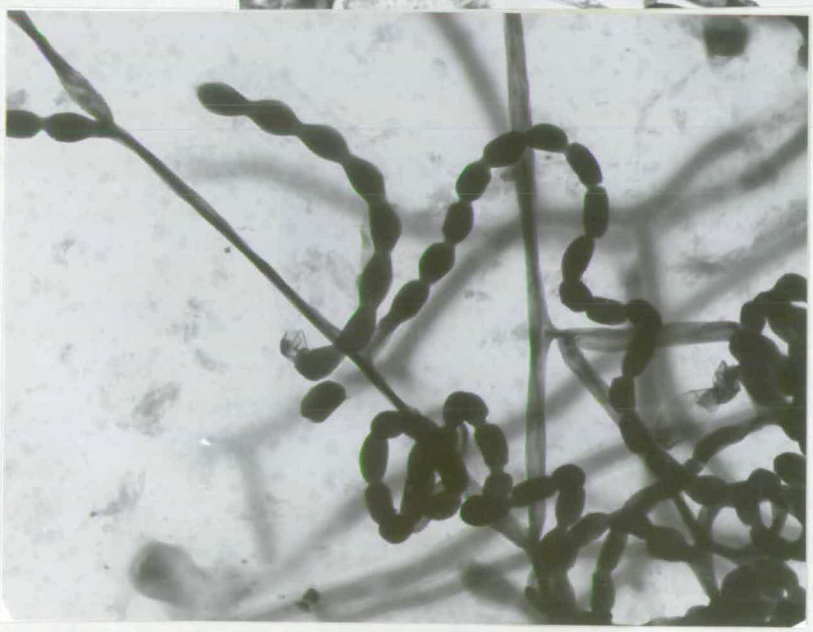


Plate 5:Electron micrographs of Streptomyces species

a)110 K.M.;hyphae and spore chains X 3,600

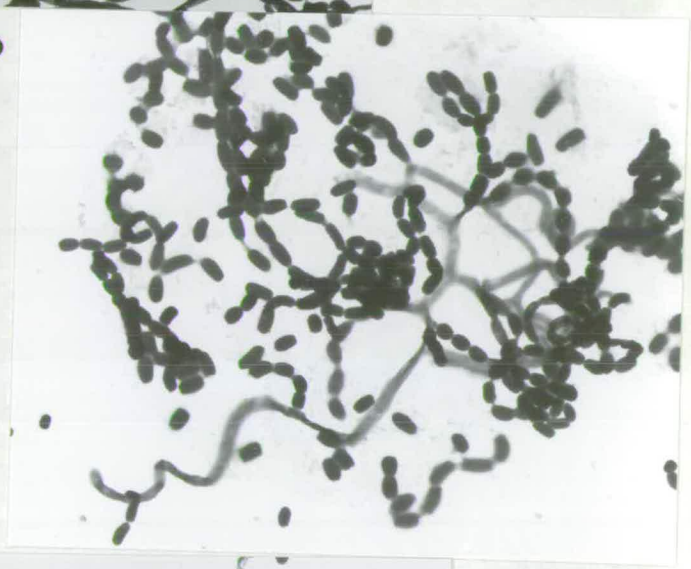
b)110 A ; " " "

c)110 A ; " " X 4,200

a



b



c

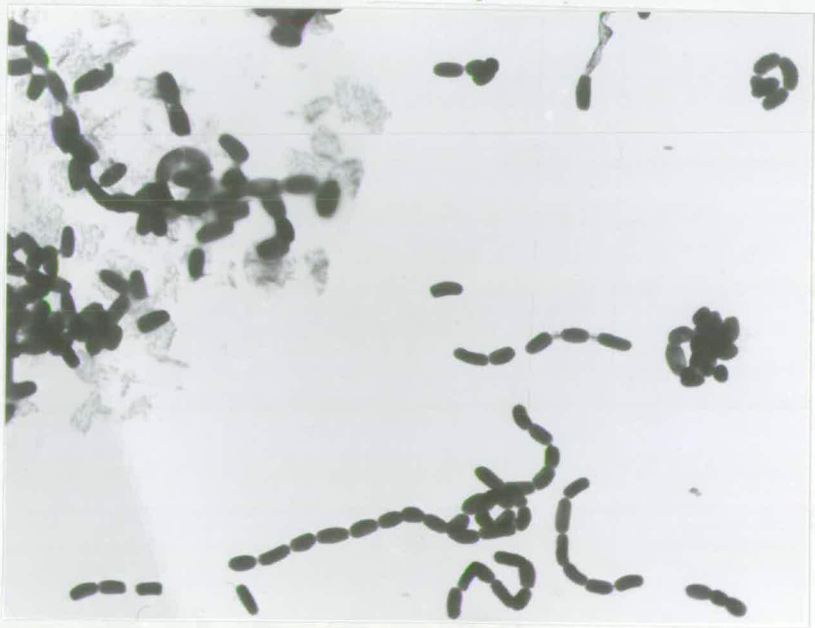


Plate 6:Electron micrographs of brown Streptomyces species

a)309 L.M.(H);spore chains X 4,200

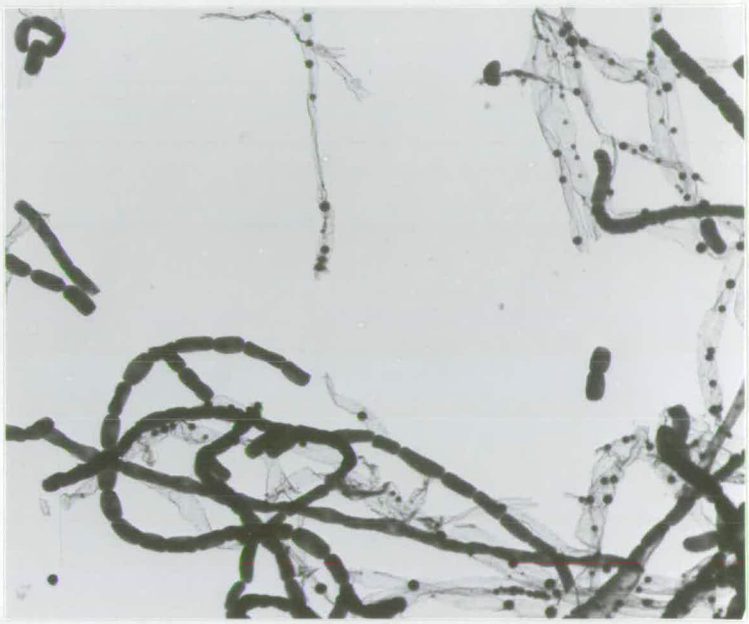
b)309L.M.(2c);hyphae and spore chains X 3,600

c)309L.M.(2c);hyphae and spore chains X 1,800

a



b



c

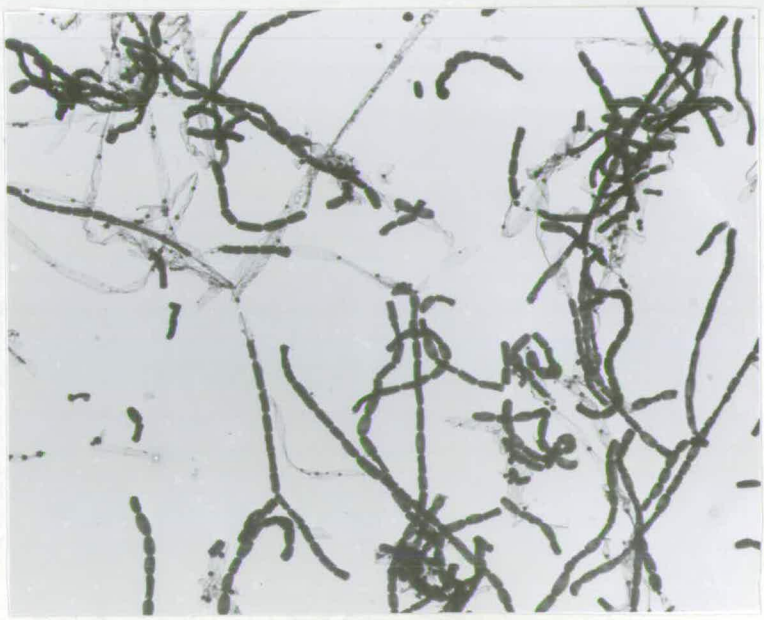


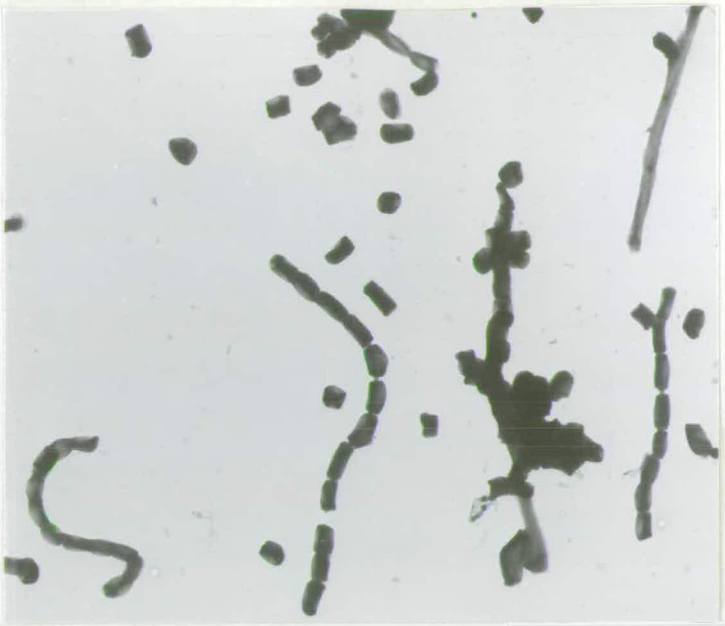
Plate 7: Electron micrographs of brown *Streptomyces* spp. and of a *Bacillus* sp.

a) 302 S.B.; hyphae and spore chains X 3,600

b) 309 ; spores X 25,460

c) 501 M.H.; *Bacillus cereus* var. *mycoides* X 3,600

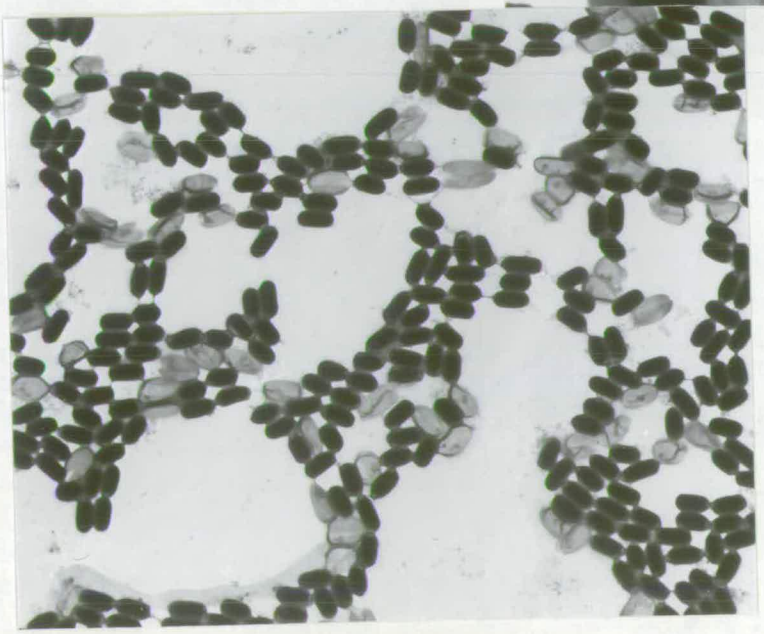
a



b



c



Discussion

In the present study, the concentrations of the components of the microflora of air in various farm buildings under different working conditions were measured. The validity of the results obtained with respect to potential human or animal respiratory disease is dependent on the accuracy of the methods used in sampling the air. The three types of apparatus used in air sampling were the Burkard seven-day volumetric spore trap (B.V.S.T.), the Andersen Sampler (Andersen, 1958) and the gravity Petri-dish (G.P.D.) all of which are described by Gregory, 1973. Their relative merits with respect to the results reported in this thesis are discussed below.

The Andersen Sampler (Andersen, 1958) was designed to assess the retention of viable propagules by various parts of the human respiratory system. Particulate matter is sifted out, according to decreasing size by means of metal plates, each containing 400 holes of uniform diameter. With each successive plate, the diameter of the holes decreases and the viable organisms collected on media below the respective plates represent those which would be expected to be retained by various parts of the respiratory tract from the nasal cavity down to the alveoli. Propagules of the dimensions of less than 1μ can be collected, free from other microorganisms by this apparatus which proved to be the most accurate in evaluating differences in the concentrations of viable propagules of both fungi and actinomycetes.

The B.V.S.T. is a modification of the automatic volumetric spore trap (Hirst, 1952) and is efficient in trapping particles above 3μ diameter (Gregory, 1973). The range of values obtained for the concentration of fungal propagules in the air by this method was of the same order as that obtained by the Andersen Sampler under similar conditions within the same environment. Values at peak concentration were higher for the B.V.S.T. and this was probably due to the fact that this apparatus traps both live and dead propagules. The rate of decrease in concentration of fungal propagules with time from peak con-

centration was similar for the two methods but the B.V.S.T. exhibited a wide range of values for the percentages of fungal propagules counted in the first fifteen minutes after peak concentrations. This could in part be due to the difficulty of counting propagules on dense regions of the trace. Other disadvantages of this apparatus relative to the Andersen Sampler are that large spore types on the trace mask the presence of smaller spore types and that particles of less than 3μ in diameter are inefficiently sampled. Both disadvantages were exemplified by the data obtained for propagules of the dimensions of actinomycetes which would be expected to be present in high concentrations at times of unbaling of hay or straw but were present in high concentrations at positions on the trace equivalent to the late evening when concentrations of other propagules were very low.

The B.V.S.T. allows enumeration of both viable and non-viable fungal propagules but the Andersen Sampler and G.P.D. technique are limited to measuring not only the number of viable propagules but which of these propagules grow on the selective media used. Non-viable propagules of organic matter can be antigenic and the B.V.S.T. may, therefore, collect material which is capable of producing an allergic response but which will not be evident on the media of plates from either the Andersen Sampler or the G.P.D. technique. It is also possible that small organic or inorganic particles seen on the trace may be a respiratory health hazard in causing physical damage to the lung tissue.

As noted by Sayer et al., 1969, the Andersen Sampler proved to be more efficient than the G.P.D. technique in isolating both the total numbers of propagules and the numbers of different species or colony types. The numbers of different colony types of actinomycetes and bacteria obtained by the G.P.D. technique, expressed as a percentage of those obtained by the Andersen Sampler was lower than the equivalent values for fungal species, reflecting the inefficiency of the G.P.D. technique in isolating smaller propagules from the air. Sayer et. al. (1969) also established that smaller fungal spore types were less commonly

isolated by the G.P.D. technique than by the Andersen Sampler at equivalent times. Despite the inefficiencies of the G.P.D. technique relative to the Andersen Sampler, the technique did give a measure of propagule concentration in differentiating between high and low concentrations of propagules although it did not appear to be accurate in assessing intermediate concentrations within one farm building. The results obtained by this method in comparing the average values for the concentrations of viable propagules in different types of Ayrshire and East Lothian farm buildings was considered to be valid as all colony counts were subject to the same limitations of accuracy.

By using the B.V.S.T. which can be considered accurate in trapping fungal propagules but not those of actinomycetes, results similar to those of Baruah (1961) were recorded. There were large fluctuations during each day in the concentrations of fungal propagules in the air of the dairy byre sampled. High values were associated with activities of animal husbandry and the largest concentrations corresponded to times of unbaling of hay or of hay and straw. Day-to-day variations in the pattern of peak evening concentrations of fungal propagules were probably due to variations in the working schedule such as the unbaling of hay or straw in several batches rather than in one operation. Unbaling in batches resulted in the presence of several peaks of intermediate concentration of fungal propagules covering a longer period of time than that of one large peak resulting from a single unbaling operation.

The highest concentration of fungal propagules obtained in this study was 7.31 million per cubic metre of air, lower than that of 16 million per cubic metre recorded by Baruah, 1961. The proportion of different spore types isolated also varied from those of that worker, in that the proportion of Aspergillus/Penicillium type spores was considerably lower and that of Mucorales type spores, higher. Other results obtained cannot be compared with those reported by him as different time intervals were involved. The highest recorded values, averaged over fifteen minute time intervals for concentrations of spores of the Mucorales type, the Aspergillus/Penicillium type and of Cladosporium spp. in the

present work were 2,094 x $10^3/m^3$, 1,505 x $10^3/m^3$ and 520 x $10^3/m^3$, respectively. In outdoor air between June and September, spores of Cladosporium spp. were found to make up 47% of the mean average of 12,500 spores/m^3 collected by a Hirst automatic volumetric spore trap (Gregory and Hirst, 1957). Spores of the Mucorales type and Aspergillus/Penicillium type made up less than 8% of the total, the completely opposite situation from that in the dairy byre. The average total value in the dairy byre was 264 x 10^3 propagules/m^3, approximately twenty times the average concentration reported by Gregory and Hirst for the Summer months. Research by other workers, including Richards (1954 a and b), using the gravity Petri-dish technique, has established that there is considerable variation between the concentrations of spores in the outside air in Summer months and concentrations in Winter. Richards (1954a) extending the work of Hyde and Williams, 1946, 1949 and 1953 confirmed that colonies of Cladosporium spp, Epioccum spp. and Alternaria spp. were more commonly isolated between June and September while Aspergillus spp. were more common in Winter than in Summer and Penicillium spp. exhibited no seasonal variations. The total counts in Winter were, however, considerably lower than those of the Summer as the colonies isolated in Winter made up only a small percentage of the total counts made over the three-year period. Colonies of Cladosporium spp. formed approximately 38% of the total isolated throughout the three years, Penicillia 9% and Aspergilli, 3%. The situation of high concentrations of fungal propagules within farm buildings is, therefore, due to the materials used in farming practice and bears no relationship to the spore-load in the outside air.

There was considerable variation in the numbers of different spore types trapped from day-to-day and this was probably a reflection of the state of microbial deterioration of the hay, straw or grain used in the byre.

During the one twenty-four hour period studied in detail, the very low average value of 19 x 10^3 fungal propagules/m^3 during the least dense off-peak region of the trace, between 00.00 hr. and 06.30 hr. and the overall off-peak

concentration of 43 x $10^3/m^3$ (over 16 hours) contrasted with an average of 706 x $10^3/m^3$ (over 8 hours) for peak periods. These figures demonstrate the removal of extremely high concentrations of fungal propagules from the environment. Lacey and Lacey, 1964, using the cascade impactor (May, 1945) obtained concentrations of 1600 million spores/m^3 for fungi and actinomycetes, of which 98% were actinomycetes,in a farm building after hay had been shaken. This concentration dropped to 10% of its original value within 20 minutes. The equivalent total fungal spore count of 20.54 million/m^3 dropped to 8% of the original value in 20 minutes, a much sharper decrease than that to between 19% and 31% of between 2.4 and 7.1 million fungal propaglues in 30 minutes in the present study. These lower figures of percentage decrease may, in part, reflect inaccurately low initial values due to the difficulties involved in counting propagules on dense regions of the trace. Using the Andersen Sampler, the decrease in concentration of fungal propagules was of the same order (ie. to 29% in 30 minutes) and within the same time interval the concentration of thermophilic actinomycetes had dropped to 18% of the original value. These percentage decreases are again dependent on the initial concentration which may not, in fact, have been the peak concentration. The difficulties involved in evaluating high concentration using the Andersen Sampler were exemplified by the large differences, in some Ayrshire farm buildings, between the numbers of colonies isolated on sets of plates containing nutrient agar with cycloheximide exposed within 45 seconds of one another and incubated at 46°C and 60°C, respectively. The very large concentration difference could not be due solely to the differences in the incubation temperature and may have reflected lack of uniformity of the microbiology of the air. Organisms may have been released in large numbers from a 'pocket' of mouldy material in the hay or grain used and as the Andersen Sampler takes in only a small proportion of the total air in the building localised 'clouds' of propagules could easily be trapped by the apparatus and give high values. Similarly, local currents of air

with small concentrations of propagules could give non-representative, low values. Even one high value does indicate that large numbers of propagules have been released into the air and are available for inspiration by both man and animals. In general, however, concentration values taken within a short time of one another were of the same order and these results were considered valid in estimating the concentrations of propagules in the air of the buildings concerned.

The methods and the rates at which propagules are removed from the air of a building are relevant to the present study. The sedimentation rate of a spore from the air is dependent on its terminal velocity which, if the spore is spherical, is a function of its diameter according to Stoke's Law (Chamberlain, 1967: Gregory, 1973). Not all spores are spherical, however, but Stoke's Law may be applied, with negligible error to ellipsoid and cylindrical spores and to those composed of up to four spheres in a row (Chamberlain, 1967). Many spore types have uneven surfaces caused either by appendages to or indentations of the spore wall which alter aerodynamic behaviour compared with that of simple spheres or cylinders. Experiments by various workers have shown that the terminal velocity of spores of Alternaria spp is 0.3 to 0.55 cm./sec., of those of Cladosporium spp is 0.07 cm/sec., for A. fumigatus spores is 0.03 cm./sec. and for Streptomyces is 0.02 cm./sec. (Gregory, 1973), in order of decreasing speed of sedimentation out of the atmosphere. The relatively high terminal velocity of larger spore types is probably responsible for the more efficient trapping of these spores by the G.P.D. technique. From the B.V.S.T. results it can be seen that spores of the Mucorales type and of Cladosporium spp. decreased in concentration more rapidly than those of Aspergillus/Penicillium type from the atmosphere of the dairy byre and this was probably a reflection of their terminal velocities (Spores of the Mucorales type have a basically spherical shape and, because of a larger diameter, would be expected to be removed from the air more quickly than those of the Aspergillus/Penicillium type). The maintenance of Mucorales type

spores in the air of the dairy byre during morning activities of animal husbandry compared with their more rapid decrease in concentration in the evening when no such activities are taking place, indicates that disturbance of the air may have prevented these spores from sedimenting out and supports the hypothesis above. Sudden, relatively large increases in the concentration of Aspergillus/Penicillium type spores at times of relatively low concentration of other fungal propagules was probably a reflection of their aerodynamic qualities as they are probably more easily made airborne by currents of air and/or by animal movement than larger spore types. Similarly, the presence of appreciable numbers of propagules of the dimensions of actinomycetes on the B.V.S.T. trace at times of low fungal concentration could have been due to the small size and low terminal velocity of these propagules. The deposition of the vast proportion of both large and small propagules was very rapid and a contributory factor may have been bulk deposition of particles, as described by Chamberlain, 1967, in which a spherical volume of air of diameter 1 metre, containing 10 mg. of particles suspended in it, could in theory, acquire a bulk sedimentation velocity of 2 cm./sec.

The removal of spores or hyphal fragments from the air of the byre was not, however, simply a function of the aerodynamic behaviour of propagules under still air conditions. Electrical charges, if any, on the surface of a spore and on those of its neighbours, as well as any charges on the surface onto which the spores were falling would have affected the rate at which spores were removed from the atmosphere. Air currents were present in the byre due to movement and to the release of body heat by both humans and animals, either by radiation or by expiration of warm air. Small particles of 3μ diameter or less move down a temperature gradient. The maximal effect of temperature gradients and therefore, the maximal deposition of small propagules by this method would probably have arisen in the boundary layers between the warm air in the building and the cold walls, as postulated by Chamberlain, 1967, for a heated room. Ventilation of the

building was aided by vents in the roof and the concentration of particles in the air was diluted by the movement of outside, cool air into the byre. This dilution effect was increased when the large doors were opened to the outside air when dung was being removed or when the doors were opened to the air of the hay and straw shed when barley feeding or bales of hay or straw were being brought into the byre. These rapid dilutions of the air were transient, however and were at times when they had little bearing on the immediate removal of large concentrations of propagules after unbaling of hay or straw.

One constant factor in the environment was the presence of 64 cattle. The tidal air ie. the volume inspired and expired by a cow at rest is approximately proportional to body weight and therefore varies from cow to cow (Dukes, 1970). The volume of air inspired by a dairy cow while lying down is approximately 3,100 ml. and while standing is approximately 3,800 ml. (Hall and Brody, 1933). The respiration rate is 27 per minute and in 10 minutes 64 animals standing in the byre would inspire 65.7 m^3 of air, approximately 5% of the total volume of air in the byre (1,341 m^3). These animals must, therefore have played a considerable part in decreasing the concentration of propagules by acting as effective trapping devices. The concentrations of fungal propagules at the level of the byre at which cows are breathing may have been different from that at the level of the B.V.S.T. orifice but nevertheless gave an indication of the concentrations with which the respirating systems of these animals had to deal. Within the eight hours of peak concentration of fungal propagules, averaging 706 x $10^3/m^3$, a standing cow would inspire 34.8 million propagules and during the sixteen hours of off-peak concentration would inspire 4.2 million propagules, making a maximum total of 39 million per day as the animal would be lying down for a considerable part of the day. Baruah (1961) estimated an intake of 11.4 million spores, excluding peak concentrations, for each cow in his study and, using the values for a three-hour continuous peak concentration of 16 million spores/m^3, calculated a total intake of 297.6 million spores per cow per day.

(This worker did not appear to allow for the decreases in concentrations of spores between peak and off-peak values and for this reason probably over-estimated the daily intake per cow from the air). The maximum concentration of fungal propagules which the animals would have to deal with in the present study was 7.31 million propagules/m^3 over a $3\frac{3}{4}$ minute time interval resulting in an average intake of 750,000 propagules per minute per cow. A factor which would probably increase considerably the intake per cow, was that the animals were eating hay and as they were very near the material and were themselves disturbing it, must have breathed in larger numbers of propagules from hay than they inspired from the general atmosphere of the byre.

The tidal volume of a human being is approximately 500 ml. and the respiration rate depends on the state of activity and health of the indivudual but is approximately 18 per minute (Druett, 1967). In one minute, at a concentration of 7.31 million propagules/m^3, the intake by the human respiratory system would be 66,000 propagules and during $3\frac{3}{4}$ minutes the value would be 247,000. Human beings were not in the building all the time but probably took in at least half of the total average daily concentration of 264 x $10^3/m^3$ in the byre ie. at least 1.7 million propagules per day. A worker could considerably decrease his daily intake of airborne material particularly that released from hay and straw, by wearing a protective mask both during and after the unbaling process in the byre. If unbaling was carried out in one operation, immediately after milking was completed and the worker then left the building, the length of time during which the mask would have to be worn would be minimal.

The concentrations of micro-organisms in the air of farm buildings is largely dependent on the initial concentrations of airborne micro-organisms in the materials such as hay and straw which are used in these buildings.

In the initial survey of Ayrshire and East Lothian small quantities of a wide range of materials were collected in 25 cc.vials. The simple, rapid

method employed consisted of blowing a small quantity of air over a previously shaken sample and thus depositing propagules via a filter funnel onto a Petri-dish containing nutrient medium. This method proved to be more valuable than direct plating which allows growth of only those organisms which are in contact with the agar medium and gives no indication of what a worker, handling the material would be likely to inhale. It is, however extremely difficult to relate the numbers of airborne organisms which would be removed under normal working conditions to those recovered by the simple method described above. There were several negative results and this method can therefore be considered valid in comparing positive results for materials subjected to it, above a certain threshold level of micro-biological content. The range of average values obtained for mesophilic fungi was from 23.5 colonies/10g. for barley samples from East Lothian to 2,984 colonies/10g. for dust samples from East Lothian. Average values for thermophilic actinomycetes ranged from zero for several groups of grain and straw to 452.7/10g. for bad hay from Ayrshire, demonstrating the wide range of values which could be obtained by this method.

The second method used in estimating the numbers of airborne propagules from samples was that of extracting a volume of air from a sealed tin containing a known weight of shaken material and passing the contaminated air through an Andersen Sampler. Again, results cannot be directly related to normal working conditions but the numbers of colonies isolated from different samples were comparable. The two sets of plates for each sample, one for thermophilic organisms, the other for mesophilic organisms were always exposed in the same order (plates for thermophilic organisms first). All results for mesophilic organisms were therefore, underestimates relative to those for thermophilic organisms as a proportion of meso philic organisms (preliminary experiments indicated approximately 40%) had already been removed by the initial sampling. Results obtained for mesoophilic organisms by this method ranged from 8 to 17.947/10g. fresh weight of hay and from 5 to 7,076/100g.

fresh weight of grain. For thermophilic organisms, values ranged from 6 to 2,959/10g. fresh weight of hay and from 5 to 2,334/100g. fresh weight of grain.

The ranges of values obtained by both the 'filter-funnel' and Andersen Sampler techniques were similar on a weight to weight basis. The method employing the Andersen Sampler, however, must be considered to have been more efficient as larger samples (approximately 50g. hay and 1,000g. grain) were tested and colonies isolated were separated out more effectively for microscopic examination and identification. Species present in small concentrations were more likely to be present in a larger sample and were also more likely to be released into the volume of air which was passed over the Andersen Sampler. The separation of propagules by the Andersen Sampler into sizes which could be related to their probable sites of impaction in the human respiratory system was of considerable value.

Using the Andersen Sampler, the lowest recorded value for mesophilic organisms from hay was approximately sixteen times the equivalent result for grain and the highest value for hay was approximately twenty-five times that for grain, on a weight to weight basis. For thermophilic organisms the ratio of the lowest value for hay to that for grain was twelve to one while the equivalent ratio for the highest values was thirteen to one. Intermediate values were also, in general, higher for hay than for grain. The ratio of surface area (ie. potential area for sporulation) to weight for hay is generally much higher than for grain and this factor may, in part, account for the relatively higher values for hay.

The microbiology of any one sample of hay, grain or other vegetable material is not representative of that of the stored product as a whole. The numbers and types of airborne propagules isolated from a sample, however, give an indication not only of the state of preservation of the material but also of what might be inhaled by human beings or animals when the material is being used in normal farming practice.

It has been well-documented that the microbiology of any stored crop

of hay or grain is largely dependent on the initial moisture content of the material and any subsequent measures taken to preserve the crop. The deterioration of stored products by micro-organisms is largely due to the self-heating process which was first examined experimentally by Miehe, 1905 and 1907 and is described by Cooney and Emerson,1964, in their account of thermophilic fungi. If the initial moisture content of plant material is sufficiently high, the metabolism of growing mesophilic micro-organisms, predominantly fungi, will lead to the production of more moisture and of heat. As the temperatures increases, thermophilic organisms predominate and temperatures of up to 70°C can be reached by the growth of thermophilic fungi, bacteria and actinomycetes. In some cases, self-heating to this extent can lead to spontaneous combustion of the crop, when subsequent chemical reactions produce even higher temperatures. Less extreme forms of self-heating cause considerable damage by reducing the nutrutional value of hay or cereal crops to livestock. The moisture content at which hay can safely be stored with negligible self-heating is 20% and for grain the value is between 12% and 15%. Although hay in the field may be considered to have a certain moisture content, there may be considerable variation within a swath of hay prior to baling and therefore, in the bales themselves. A similar situation can exist in grain and if 'pockets' of sufficiently high moisture contents are present, 'hot-spots' - localised regions of self-heating, may be formed. These hot-spots may be contained by a lack of oxygen but, if not, may lead to more extensive damage of the stored crop as can local pockets of self-heating in hay. Preservation of grain is further complicated by the fact that it is usually still alive when stored and at a suitable temperature and comparatively low-moisture content could release sufficient moisture by respiration to start self-heating. Self-heating is an aerobic process, however, and the production of carbon dioxide by the respiring grain would prevent growth of micro-organisms unless an adequate source of oxygen was

available.

Hay and grain can be preserved by a variety of methods. Grain can be sealed off from an oxygen source either by storing it in polythene bags containing ½ cwt. or 1 cwt., or on a large scale by placing it in a silo within a large bag of butyl sheeting which lines the sides of the silo and completely excludes air. As material is removed from the bottom of the silo the sheeting falls in on top of the remaining grain, maintaining a seal throughout the Winter season. The resulting anaerobic conditions prevent respiration of the micro-organisms which are largely responsible for self-heating. Grain is also stored in cement-sided roofed silos or in metal-sided silos with butyl sheeting down the sides, both with a seal of straw and/or tarpaulin on top of the grain. An initial accumulation of carbon dioxide in this system prevents subsequent growth of aerobic organisms but this and the previous form of storage can go seriously wrong if any oxygen is allowed in, either by damage to the butyl sheeting or by disturbance of the seal. The mycelial mat formed during aerobic respiration can lead to 'bridging' of the grain and difficulties in removing it from the storage system. In some cases, this situation is associated with very high concentrations of carbon dioxide which can be fatal to workers attempting to remove the 'bridged' grain. The popularity of this method is largely due to the fact that moist grain is more suitable for bruising and that moist bruised grain is more easily digested by animals. Propionic acid ('Propcorn') added uniformly to moist grain prevents growth of micro-organisms and kills the grain itself. Inefficient application of this preservative can give rise to 'hot-spots' in grain. Artificial drying of grain to reduce the initial moisture content is another means of preservation but it is again difficult to ascertain whether all the grain is of a sufficiently low moisture content to prevent fungal growth. As no precautions are taken to prevent oxygen from being in contact with such grain, which is usually stored in bins or jute bags, 'hot-spots' may be formed which could lead to damage of part or all of the crop.

Baled hay can be dried by artificial means to reduce moisture content prior to storage but the application of preservative to hay is a considerably more difficult task than application to grain due to the lack of uniformity in density and moisture content of the material and to mechanical difficulties.

Several workers have investigated the microbiological content of hay and grain with respect to types and species of organisms which are present under good storage conditions and those which predominate under conditions of deterioration of the materials due to self-heating. As the dusts from vegetable products which have shown evidence of moulding are implicated in causing farmer's lung disease and also bovine farmer's lung disease, the numbers and types of organisms isolated from such dusts are of considerable importance.

Gregory and Lacey (1963a) using a wind tunnel in conjunction with a cascade impactor (May, 1945) established that samples of 'good' hays with no evidence of self-heating release fewer spores (up to 3 million per g. dry weight) than those of 'mouldy' hays which showed evidence of self-heating and released between 5 and 250 million spores per g. dry weight. These workers also identified the predominant fungal species isolated from 'good' hays and those from self-heated hays. By examining experimental batches of hay baled at different moisture contents, Gregory et al.,1963, related initial moisture content at the time of baling to the predominant species present under storage conditions. Festenstein et al., 1965, using the artificial situation of hay and grain samples of a known moisture content stored in aerated Dewar flasks came to the conclusion that "from a knowledge of the Aspergillus spp. and actinomycetes present on a sample of mouldy hay, the initial moisture content and maximum temperature during self-heating can be assessed". According to their findings, a moisture content of 26% correlated with the predominance of A. glaucus, of 28-29%, with A. versicolor and Scopulariopsis brevicaulis,of 31% with A. nidulaus, Absidia sp. and Streptomyces fradiae, of 40% with

A. fumigatus and Humicola lanuginosa, of 47% with H. lanuginosa, Thermopolyspora glauca and Thermopolyspora polyspora (Micropolyspora faeni) and of 57% with Micromonospora vulgaris (Thermoactinomyces vulgaris) and Paecilomyces variotii. These results were similar to those of Gregory et al., 1963, but they also established that the situation within an entire stack stored at a high moisture content was very different from that for small samples or for small numbers of bales. Hay in the centre of the stack heated, becoming brown and acid but the organisms responsible were predominantly bacteria with fungi present only in the outer mouldy layer of the stack. Hays baled at the low moisture content of 15% - 17% released relatively few colonies but a wide variety of species. In one sample colonies of Cladosporium spp., a 'field' fungus were predominant.

Festenstein et al.,1965, also examined grain stored at high moisture contents in aerated Dewar flasks. The species isolated differed slightly from those of hay. Barley stored at 33% to 35% initial moisture content, self-heated to produce colonies of A.flavus, Humicola lanuginosa, Penicillium spp. and Absidia spp. Barley with an initial moisture content of 40% contained many Aspergilli, notably A. fumigatus, Mucor sp. and Humicola lanuginosa. From an oats sample which self-heated from an initial moisture content of 37%, A. fumigatus, A.nidulans and an Absidia sp. predominated. All four grain samples examined yielded Streptomyces fradiae, Thermopolyspora polyspora (Micropolyspora faeni) and Micromonospora vulgaris (Thermoactinomyces vulgaris) in large numbers, except one which yielded only Streptomyces fradiae in large numbers. Lacey (1968) recorded values of 5.9 to 81.5 million spores of actinomycetes and 0.5 to 26.7 million spores of fungi per gram dry weight of grain in samples from farms associated with bovine respiratory disease. Lacey (1965) recovered colonies of Aspergillus fumigatus, A. flavus, A.nidulans, A. terreus, A. candidus, A. glaucus, Mucor pusillus, Absidia corymbifera, Humicola lanuginosa, Thermoactinomyces vulgaris and Thermopolyspora polyspora from the surface layers of mouldy grain during moist barley grain storage.

Differences in the proportion of the various species was attributed to variation in moisture content. Penicillium spp. and yeasts predominated in the bulk of the grain present under the mouldy upper layers. (Lacey, 1971).

Indications of the conditions under which some of the storage fungi isolated by Lacey (1965), had grown are given by the findings of other workers who have examined the microflora of stored grain. The sampling methods used by these workers were those of plating non-sterilised or surface-sterilised grain onto agar media or washing the grains and plating dilutions of the washings. Results from these methods, therefore, give only an indirect measure of potential respiratory hazard by relating stages of the self-heating process to the dominant fungal species isolated.

Christensen (1957) and Christensen and Kaufmann(1969) have describd the relationship between fungal species isolated from dried, stored grain in North America and the moisture content at which the grain had been stored. Field fungi present at the time of storage were mainly species of Alternaria, Fusarium and Cladosporium. After storage at 14.5% to 15% moisture content the A.glaucus group predominated and above 15%, A. candidus (at 16% to 17%), A. flavus (at 18% to more than 19%), A. versicolor, A. tamarii and Penicillium spp. were quoted by Christensen (1957) to be those most commonly isolated. Wallace and Sinha (1962) examined 'hot-spots' in dried, stored grain, some of which reached the temperature of 53^{o}C during the Canadian Winter. Penicillium spp.were the most abundant. A. flavus and A.fumigatus were also associated with wheat which had heated and the species A. flavus, A. fumigatus and A.versicolor were associated with self-heated oats. A.candidus, A. glaucus and A.ochraceus were isolated, but less frequently. These authors also associated the presence of Streptomyces spp.with rapid growth of storage fungi and the resulting high temperatures.

Mulinge and Chesters (1970) investigated the storage fungi of moist grain in a cement-sided silo in Northern England. They established a succession of

dominant species from the field fungi, Alternaria spp.,Fusarium spp., Cladosporium spp. and Mucor spp. at the time of storage to species of Aspergillus, Penicillium, Absidia and yeasts, two months after storage. Penicillium spp. and yeasts were finally the dominant species. A. candidus was most commonly isolated from the straw used as a seal for the grain. A. glaucus and A.versicolo were dominant in the 'drier' samples of grain and in samples which showed evidence of self-heating, A. fumigatus, A. terreus, A.flavus, Mucor pusillus and Dactylomyces crustaceus were predominant.

Flannigan (1969) examined barley which had been harvested in Eastern Scotland during 1967, dried to 12% to 15% moisture content and stored for one month. This worker established that the dominant groups or species present were bacteria, actinomycetes, Absidia corymbifera, Alternaria tenuis, Aspergillus fumigatus, A. glaucus (group), Cladosporium spp., Epicoccum nigrum and Penicillium spp. Colonies of Aureobasidium pullulans, Botrytis cinerea, Cephalosporium sp., Fusarium avenaceum, Mucor pusillus and Sporobolomyces roseus were also frequently isolated. Alternaria tenuis, Penicillium spp. and A. glaucus were most commonly isolated from kernels examined and from the work of previous workers this would indicate that the grain had undergone relatively little self-heating, particularly as there was such a large proportion of field fungi which, according to Wallace and Sinha (1962) was found to be negatively correlated with self-heating. In a further paper, Flannigan (1970) reported the species isolated from samples of dried wheat, barley and oats collected in Eastern Scotland within six weeks of harvesting in 1968. Again, Penicillium spp. and A. glaucus were predominant. Oats, in general yielded a wider range of species on the basis of the percentage of kernels infected per sample, than either wheat or barley.

In the survey conducted in the three areas of Scotland - Ayrshire, East Lothian and Orkney, it was established that there was a correlation between the prevalence of farmer's lung disease and a less high standard of preservation

of farm products, measured by the numbers of thermophilic organisms isolated from environments and from random samples of farm products.

Air-sampling by the G.P.D. technique of farm buildings, outhouses and greenhouses in Ayrshire and East Lothian established that there were generally higher values for mesophilic and thermophilic actinomycetes and fungi in Ayrshire environments than in equivalent East Lothian environments. It was also demonstrated that, on average, higher values were obtained in buildings in which hay, straw or grain were handled than in others. In Ayrshire dairy byres the numbers of thermophilic organisms isolated were considerably higher than in East Lothian cattle-courts. As the majority of the population in Ayrshire was employed on dairy farms and in East Lothian was employed on arable stock farms, these buildings were representative of the working environments of the bulk of the population interviewed with respect to farmer's lung disease symptoms. The high prevalence of the disease in Ayrshire (86 per 1000) correlated with high numbers of thermophilic organisms not only in environments but also in the hay and straw samples examined. Equivalent East Lothian hay and straw samples realised lower values for thermophilic organisms and there were few bad samples of hay in the random collection made. This indicates a higher standard of preservation of materials in East Lothian and therefore less evidence of self-heating of farm products in a county with a low prevalence of farmer's lung disease (23 per 1000). This association of farmer's lung disease with self-heated products was further reinforced by the high numbers of thermophilic organisms isolated from hay and dust samples from Orkney which had a farmer's lung disease prevalence of 86 per 1000. The types of materials and environments sampled, which were implicated by the farmer's lung disease patients in causing their symptoms were those with the highest numbers of thermophilic organisms. There was, unfortunately, no direct correlation between specific environments and products and particular patients but, on a general basis, indicated the main sites and sources of allergens. Hay and straw were the main hazards implicated in Ayrshire and Orkney. Grain was also a

substantial hazard in these two areas but in East Lothian was the main hazard in all four cases of the disease. It was of interest that the only East Lothian samples with high values for thermophilic actinomycetes were bruised barley and dusts from bruising-machines, implicating self-heating of grain as a source of these organisms in bruising sheds. The bulk movement of grain at harvest-time was the main activity cited by three of the four East Lothian cases in causing disease symptoms. Samples of grain were not obtained at this time of year but it was observed by some of the workers concerned that grain from the field released a considerable amount of dusty material and that on some occasions, prior to drying and/or storage, heating of a cart-load or heap of grain could take place within twenty-four hours. This phenomenon was observed on one farm and to the touch, the grain had heated considerably, whether by respiration and/or growth of organisms was unknown. It would, therefore, be of value to establish what was the microbiological content of the air-borne dust produced during such activities as augering or shovelling large amounts of newly-harvested grain. Typical disease symptoms such as shivering and feverishnness were noted by some workers several hours after being exposed to such dust.

The association of self-heated products with farmer's lung disease was again illustrated by the results obtained in a survey of farmer's lung disease (FLD) and non-farmer's lung disease (non-FLD) farms in Ayrshire. The microbiological content of hay, grain and dust samples and of the air of dairy byres and bruising-sheds in which these materials were handled is discussed below.

From hay samples, the species of mesophilic fungi most commonly isolated were those of the Aspergilli, apart from one sample in which mesophilic actinomycetes predominated. Species present in relatively large numbers in 'bad' hay samples which were superficially mouldy and showed evidence of self-heating were Aspergillus fumigatus, A. glaucus, A. nidulans, A. terreus, species of the Mucorales and Penicillium spp. As discussed

earlier these species have already been associat ed by previous workers with self-heated products. On the basis of the findings of Festenstein et al., 1965, with respect to these species, the approximate initial moisture content of bad hay samples ranged from 26% to 40%. Species isolated from good hay samples from FLD farms and non-FLD farms indicated that the highest moisture content at which they had been baled was probably 28%-29%. On the basis of the predominant thermophilic organisms isolated from hay samples, three from FLD farms with concentrations of between 2,450 and 6,143 colonies of M. faeni per gram fresh weight indicated a probable initial moisture content of 47%. (These values are not high when compared with the millions of spores isolated from samples in a wind tunnel (Gregory and Lacey 1963 a and b) but within the range of values obtained by the method used, were very high). One sample with high numbers of M. faeni propagules also released appreciable numbers of the 401 type colony probably Thermopolyspora glauca described by Corbay et al.,1963 and also cited by Festenstein et al. (1965),if present in large numbers,as being associated with initial high moisture contents of hay. One other sample from an FLD farm with low values for M. faeni yielded appreciable numbers of another thermophilic actinomycete associated with self-heating, Thermoactinomyces vulgaris. These four last-mentioned hay samples were from farms where hay was considered to be a major hazard by FLD patients. No other hay samples gave values of the same order for thermophilic organisms but two yielded low numbers of colonies of M. faeni.

From the air of the dairy byre of farm B.P., high concentrations of propagules of Aspergillus fumigatus, A. nidulans, species of the Mucorales and yeasts were associated with high concentrations of the same species (apart from A. nidulans) in the bad hay sample from the same farm. No colonies of M. faeni(in contrast to high values in the hay) were isolated from the air of the byre but those of the thermophilic 401-type were.

Spreading colonies of bacteria may have inhibited or overgrown M. faeni on the environmental plates. On other farms differences between the microbiology of the hay samples and that of the air of dairy byres were even more marked than those on B.P. but again, from four FLD farms high numbers of thermophilic organisms, notably M. faeni were isolated, together with relatively high concentrations of A. terreus from one of these FLD environments and thermophilic fungi from another.

Anomalies between the microbiology of hay samples and that of the air of the buildings in which hay from the same crop was being used can be attributed to variation within the individual crop, indicated by the good and bad hay samples obtained from FLD farms. From non-FLD farms, obviously bad hay was not available but must have contained appreciable numbers of thermophilic organisms on two non-FLD farms (M.T. and M.F.) as high numbers of these organisms were isolated from the dairy byres of both. The sample/s taken at each farm were only small fractions of the crop as a whole and air-sampling represented only a small volume of air. Both types of sampling were random, however, and although not as valid as replicate sampling would have been, may be viewed as indicators of the microbiological content of the buildings and materials concerned.

Differences between the numbers of propagules and species isolated from whole barley compared with those from the air of bruising-sheds were less marked than those between hay and the air of dairy byres. Penicillium spp. and yeasts and occasionally Geotrichum spp. were the dominant species in the majority of air samples from bruising-sheds and from samples of whole and bruised grain and dust. A wide range of values was obtained for all types of sample and there was no correlation between the numbers of mesophilic organisms (notably Penicillium spp., associated by previous workers with self-heating) and those of thermophilic organisms. On a qualitative basis, however, it was established that high numbers of thermophilic organisms from whole barley

samples from two FLD farms, were associated with the mesophilic species A. candidus from one sample and A. fumigatus from another. It was also of interest that the only bruised barley samples and dusts to yield relatively high values for thermophilic organisms were from these two farms on which grain-bruising was implicated in causing FLD symptoms. On one other FLD farm on which this activity was considered to be the sole cause of disease symptoms, appreciable numbers of thermophilic organisms were isolated from bruised barley and dust.

It is doubtful whether the presence of thermophilic organisms in bruised barley was due to growth and sporulation of these organisms on the material, as all samples were refrigerated within a few hours of removal from the farm. Some Penicillium spp. can grow at low temperatures but other organisms and particularly thermophilic organisms were probably in the form of propagules which had settled out from the air during and after the bruising process. Under normal working conditions these propagules could have been inhaled by both workers and animals. In the process of bruising, large concentrations of dusty airborne material are produced both by movement of grain from its storage position and by vibrations of the machinery during the process itself. This dust gathers on machinery, walls and other surfaces of the shed. By examination of dust samples collected in Orkney, Ayrshire and East Lothian, it has been shown that some contain large concentrations of thermophilic organisms. The worker is therefore in contact with two sources of potential respiratory hazard - dust from the grain itself and that which has arisen from previous activity. Values for the numbers of both mesophilic and thermophilic organisms isolated from the dust samples of Ayrshire farms were surprisingly low compared with the values for whole barley and air samples but it may be that dead propagules which could still constitute a health risk, were present in high concentrations. In contrast to unbaling of hay, with a comparatively rapid increase, short peak period and decrease in propagule concentration,

bruising of grain may be considered to maintain high concentrations of propagules for a longer time as a new source of grain is continuously being passed through the machine. Although, as observed earlier, the concentrations of propagules released per unit fresh weight of hay were greater than for grain, the weight of grain processed in one operation is generally larger than the weight of hay in one unbaling operation. Concentrations of up to 2,244 thermophilic actinomycetes and bacteria/m^3 (2,020 colonies of M.faeni) were recorded during grain bruising and of up to 1,670 thermophilic actinomycetes and bacteria/m^3 (1,453 colonies of M. faeni) after unbaling of hay. Workers often leave the building while bruising is taking place but if the grain is being placed in bags, a worker will have to remain in close contact with the dust for upwards of fifteen to twenty minutes. Although grain bruising may only take place every second or third day in general farming practice, compared with twice-daily unbaling of hay, the overall intake over a period of weeks is possibly comparable.

The main factor involved in determining the quantity and nature of airborne material inhaled by workers is not the farming activity involved but the state of preservation of the material used. Farmers, in general, are very conscious of the loss in nutritional value of grain and hay due to microbial deterioration and many of those interviewed took steps to prevent such deterioration of grain by one or more of the methods discussed previously. During the survey of FLD and non-FLD farms in Ayrshire in March/April 1972, farmers observed that the previous hay-making season (1971) had been exceptionally good and considered that it would be difficult to find bad hay samples. Microbiological values for samples taken during this part of the survey can therefore be regarded as minimal, on a general basis. The hay-making season in 1970 had been a poor one and results for the earlier phase of the survey in Ayrshire were probably comparatively high. From these observations it would appear that the standard of preservation of hay was, in large part, a reflection of the

rainfall during the hay-making season. In view of this, it was surprising that so few farmers either dried their hay artifically or alternatively, made silage to combat the probable vagaries of the weather. The main drawback in both these processes is that they require capital outlay. Even if hay or grain deteriorates considerably due to self-heating, the farmer or his workers could protect themselves from dust by wearing masks. Apart from farmer's lung patients who were forced to wear masks, only a small proportion of these interviewed wore them and, in general, only a surgical face-mask, which gives insufficient protection against organisms of the dimensions capable of penetration to the alveoli, was worn.

The involvement of propagules of the dimensions of actinomycetes or bacteria is substantiated by the fact that a high concentration of thermophilic bacteria and actinomycetes, notably Micropolyspora faeni, was the one factor common to Ayrshire farms on which cases of farmer's lung disease were found.

A relationship has been established between clinical symptoms of farmer's lung disease and sensitisation of individuals to farmer's lung hay (F.L.H.) antigen. The type of hay which produces this antigen has the characteristics of having self-heated due to an initial moisture content of 30% or more, a final pH (approximately 7.0) which is higher than that in other types of hay and a characteristic microflora (Gregory et al., 1964; Festenstein et al., 1965). Antigenic extracts of M. faeni have been shown to contain a large proportion of the antigens present in F.L.H. and to yield the A, B and C arcs characteristic of F.L.H. antigen during immunoelectrophoresis (Pepys et al., 1963). They demonstrated that extracts of another thermophilic actinomycete, T.vulgaris, reacted only in the C region in this serological test and was positive to only a proportion of those sera positive to extracts of M.faeni and/or to F.L.H. The F.L.H. production by particular hay samples stored in aerated Dewar flasks was shown to be related to the

numbers of colonies of M.faeni present in the samples and both reached a peak in samples with an approximately 40% initial moisture content and which had reached a maximum temperature of approximately 60°C (Festenstein et al., 1965). With increasing moisture content above 40%, the amount of F.L.H. decreased. In self-heating grain the pattern of F.L.H. production was similar.

Pepys et al. (1962) established that 80% of patients with farmer's lung disease reacted to F.L.H. antigen and Pepys and Jenkins (1965) quoted figures of 87% positive to extracts of M. faeni.

In the pilot survey of East Lothian, Orkney and Ayrshire (Grant et al., 1972) only 43% of patients classified as having clinical symptoms of farmer's lung disease reacted to standard F.L.H. antigen when tested by the routine double-diffusion method employed by the Public Health Laboratory Service. Situations similar to those described by Hapke et al., (1968) may, in part, be responsible for this low positivity to F.L.H. antigen in clinically diagnosed patients. These workers established that 53% of patients in the chronic stage of the disease were serologically positive to M. faeni, compared with 100% in the acute phase. They also demonstrated that this positivity in the acute stage could vary in titre within days of initial testing and could completely disappear and return again during intervals of between ten days and eleven months.

These findings cast doubt on the validity of using a serological test involving F.L.H. and extracts of M. faeni as an aid in diagnosis of farmer's lung disease (F.L.D.). A correlation between prevalence of F.L.D. and the numbers of thermophilic organisms isolated from farm buildings and products was established in the survey of Ayrshire, East Lothian and Orkney but as information on the microbiology was slight, it was possible that the concentration of certain fungal species and/or actinomycetes other than M. faeni or T. vulgaris might also correlate with disease in particular farm environments. In the second phase of the survey, in Ayrshire, a more detailed examination of the microbiology of selected farms, with and without F.L.D., was made.

Extracts of organisms, many of which were involved in the self-heating process and which included species of Aspergillus and also thermophilic actinomycetes, fungi and bacteria were tested against sera from personnel on these farms. There were very few reactions to any of these antigenic extracts by sera from 11 F.L.D. patients or from the 27 non-FLD personnel and these few reactions did not appear to follow any pattern with respect to farmer's lung disease. The organisms which were cultured cannot be said to represent all the possible range of species or strains of species which were present in farm buildings or products such as hay or grain. Gregory and Lacey, 1963a established that only one in a thousand of spores collected from hay samples by the cascade impactor were recovered by the Andersen Sampler, due to inviability of the spores, inappropriate media or inhibition by other organisms. Sources of allergen other than culturable organisms may also be present.

Extracts of hay, grain and dust samples from the Ayrshire farms surveyed were tested against sera and their reactivity compared with that to F.L.H. antigen and to extracts of M. faeni and T. vulgaris.

Although no biochemical analysis of antigens from farm products was made in the present study, it was assumed that they corresponded to those of Pepys et al., (1963) who used phenol saline to extract F.L.H. antigens and were mainly glycoprotein in constitution as reported by Laberge and Stahmann (1966). There was a range of reactivity of the 38 sera to F.L.H. antigen and extracts of M. faeni. Six sera which reacted strongly to F.L.H. in the double diffusion test also reacted strongly to culture filtrate extracts of M. faeni (Lacey strain) in double-diffusion, immunoelectrophoresis and immunoelectro osmophoresis tests and also in the fluorescent antibody test in which whole cells of M. faeni were employed. These highly reactive sera also gave positive results in double-diffusion tests to a high proportion of extracts of strains of M. faeni isolated from the farms surveyed and to extracts of hay and grain. Other sera showed a less consistent pattern of reactivity and two FLD

sera, one with no reactivity to F.L.H. or M. faeni (serum 13) and the other with weak reactivity to F.L.H. and in the fluorescent antibody test (serum 28) reacted strongly to local strains of M. faeni. They did not, however, react with the extracts of the type culture of this organism although all the cultures were morphologically similar to the type culture and produced characteristic arcs in A, B and C regions to a reactive serum. This apparent specificity may only be a reflection of the concentration of antigen in the respective extracts in that the concentration in F.L.H. and the M. faeni type culture extract might have been too high, giving inhibition of reactivity in sera 13 and 28. This would not, however, fully explain the similar specificity of some other sera which reacted to only one or two of these six local strains, which were all cultured and extracted in the same manner, used at the same concentration and gave similar patterns of reactivity to a test serum.

The distribution of sensitisation of FLD and non-FLD personnel to F.L.H. and M. faeni was similar. There were three strongly reactive sera in each group and 52% of sera from personnel on FLD farms and 54% of those on non-FLD farms were positive in one or more tests to F.L.H., to M. faeni extracts and to the fluorescent antibody test. On the basis of these findings it was, therefore, impossible to differentiate serologically between diseased and non-diseased personnel as on FLD farms, 67% of sensitised individuals were also symptomatic whereas none were so on non-FLD farms. It is, perhaps, significant that the proportion of sensitised personnel with disease symptoms correlated with significantly higher concentration of M. faeni in the air of buildings and in farm products and therefore with a lower standard of preservation of hay and grain. It may be that disease is caused by a larger intake of antigen and that a lower level causes sensitisation of individuals without symptoms. The occurrence of a larger or more potent source of antigenic material of undefined content on FLD farms was indicated by the fact that the

extracts of hay, grain and dust from these farms gave reactions with a larger number of sera than equivalent samples from non-FLD farms. This reactivity was not equivalent to that of F.L.H. antigen but was related to a less high standard of preservation of hay and grain. This, again, indicated a random distribution of sensitisation similar to that to F.L.H. and M. faeni but limited to non-FLD sera from the two types of farm and, therefore, not associated with disease symptoms.

The highly reactive sera were relatively non-specific in their strong positivity to extracts of hay, grain and dust and this reactivity did not relate to the microbiological content or apparent state of perservation of the materials tested. Not all antigenic components of mouldy hay can be attributed to M. faeni or to fungal antigens (Pepys et al., 1964). Other, undefined antigens may have caused reactions in sera from F.L.D. patients which were unrelated to those of M. faeni or fungi or perhaps to those of F.L.H. itself. Only two FLD sera, other than the three highly reactive sera, were strongly positive to any extracts of hay, grain or dust, but all sera (both FLD and non-FLD) gave weak reactions to between 1 and 15 of the sixty extracts tested.

There were, therefore, four levels of sensitisation.

1) a background level of weak sensitisation of all personnel in varying degrees to antigenic components of hay, grain or dust.
2) a level of stronger sensitisation to materials such as hay, grain and dust of a proportion of non-FLD personnel and of a smaller proportion of FLD personnel
3) strong sensitisation, in varying degrees, to antigenic components of F.L.H. and M. faeni, common to an equivalent proportion of both FLD and non-FLD personnel
4) very strong reactivity to F.L.H., M. faeni and to extracts of hay, grain and dust by a small number of both FLD and non-FLD personnel.

The latter two types of sensitisation were associated with both healthy and diseased individuals. Pepys and Jenkins, 1965, stated that 15%to 21% of healthy farmers reacted to extracts of mouldy hay containing M. faeni. In the present study, 30% of 'healthy' individuals and 55% of diseased individuals ie. those who had clinical symptoms, reacted with F.L.H. in the double-diffusion test. However, 11% of healthy individuals and only 27% of diseased individuals gave strong reactivity to F.L.H., M. faeni and hay, grain and dust extracts. The pattern of arcs formed in the A, B and C regions during immunoelectrophoresis was not related to apparent disease symptoms except that serum from one non-FLD individual reacted only in the A and B regions. It may be that non-FLD individuals with highly reactive sera had had disease symptoms which were not severe enough to be considered as such by them or that this reactivity is merely a measure of exposure to the antigens concerned. The fact that a large proportion (55%) of all individuals tested were sensitised in varying degrees to F.L.H. and/or to M. faeni indicates contact of the individuals concerned with these antigenic components and is therefore evidence of self-heating of products on the farms studied. At the time when samples were taken, levels of antigen were higher in FLD farms than in non-FLD farms. There is, however, yearly variation in the degree of self-heating of products on a particular farm as well as variation within any one crop. In previous weeks, months, or years, high dosages of antigen could have been present in the non-FLD farms, causing sensitisation of non-symptomatic individuals similar to that in FLD patients. It may, however, be that a lower background level of F.L.H. or M. faeni antigen may cause a sequential increase in sensitisation but not disease symptoms. All such sensitisation does, however, indicate some degree of self-heating of farm products on all farms and therefore an overall level of preservation of materials which is conducive to disease. The high figures for prevalence of farmers lung disease in Ayrshire (86/1000) would seem to indicate that the levels of preservation of

materials in the farms studied was representative of those in the county as a whole. The association of F.L.H. and M. faeni with disease is supported by the fact that the diseased group has the highest proportion of positivity to antigenic fractions of M. faeni and to F.L.H. It is, however, possible that other, as yet undetermined antigenic moieties, related to microbiological deterioration of vegetable products and, therefore, directly or indirectly associated with M. faeni or F.L.H. are involved in the aetiology of farmer's lung disease and could show a sharper differentiation between symptomatic and non-symptomatic persons than that demonstrated by antigens employed in the study discussed above.

Frankland and Gregory (1973) reported that extracts of ascospores of Didymella exitialis, a fungus which grows on barley leaves, were positive in skin tests to a proportion of patients with seasonal rhinitis and/or asthma. They also claimed that this fungus may cause a type III reaction, resulting in a form of allergic alveolitis as precipitating antibodies to extracts of the fungus were found in one patient. It is possible that high concentrations of this or other invasive field fungi could be responsible for the symptoms of a type III hypersensitive reaction, reported by workers at harvest-time, but this is a very tentative suggestion.

Farmer's lung disease is as much an agricultural as it is a medical problem. It would, in theory, be possible to control acute disease and prevent new cases by achieving an overall high standard of preservation of cereal crops and hay or by the use of silage in place of the hay crop. Even without the financial expenditure that these measures would entail, the use of efficient masks (Gourley and Braidwood, 1970) by workers in contact with dust from farm products would restrict the intake of antigen and aid in controlling disease. The implementation of these steps is, however, dependent on adequate incentive and in many cases, it may be that control measures will only be taken after the disease has manifested itself. More understanding of the aetiology of farmer's lung disease and a better method of differentiating

between healthy and diseased individuals than is feasible using the present serological tests would therefore be of considerable value.

APPENDIX A.

Agar media

Malt

20 g. malt extract (Oxoid)

20g. Davis Standard Agar

1 l. distilled water.

Half-strength nutrient with cycloheximide

6.5 g. nutrient broth (Oxoid)

500 mg. cycloheximide

20 g. David Standard agar

1 l. distilled water

Half-strength nutrient with cycloheximide and novobiocin

6.5 g. nutrient broth (Oxoid)

50 mg. cycloheximide

25 mg. novobiocin

20 g. David Standard agar

1 l. distilled water

Glycerol asparaginate agar

10.0 ml. glycerol

1.0 g. $K_2 H PO_4$

1.0 g. sodium asparaginate

20 g. David Standard Agar

1 l. distilled water

All media were sterilised by autoclaving for 15 minutes at 15 lbs. per square inch.

Cychoheximide and novobiocin were added to cooled agar after being sterilised by passage through a millipore filter of 0.22 μ. pore size.

APPENDIX B.

The Andersen Sampler (Figure 25) was run at an air-flow of 25 l./min., calibrated by a flow-meter before each set of samples was taken. The 9 cm. Petri-dishes used in the Sampler were filled with molten agar to the level recommended by the makers for efficient sampling. Plates were left at least overnight before use. After air had been passed over the agar medium, the lids of the Petri- dishes were swabbed with ethanol before being replaced, to prevent contamination. Several tests were made between times of sampling of air and of vegetable products and on all occasions contamination was zero or negligible.

Counting of colonies

The numbers of colonies of organisms were counted after 48 hours using either the positive hole method or the microscope method (Andersen, 1958). In the former, the number of areas (corresponding to the 400 holes in the plate above) which contain colonies were counted. Using the conversion table of Andersen, 1958 the number of 'positive holes' was converted to the number of propagules which, allowing for aerodynamic effects, would yield that number of positive holes. If, as seen in Figure 26, all the 'holes' contained colonies then the microscope method was employed. The number of colonies in approximately six to ten 'holes' were counted and the average number per 'hole' was calculated and then multiplied to give the total number per plate.

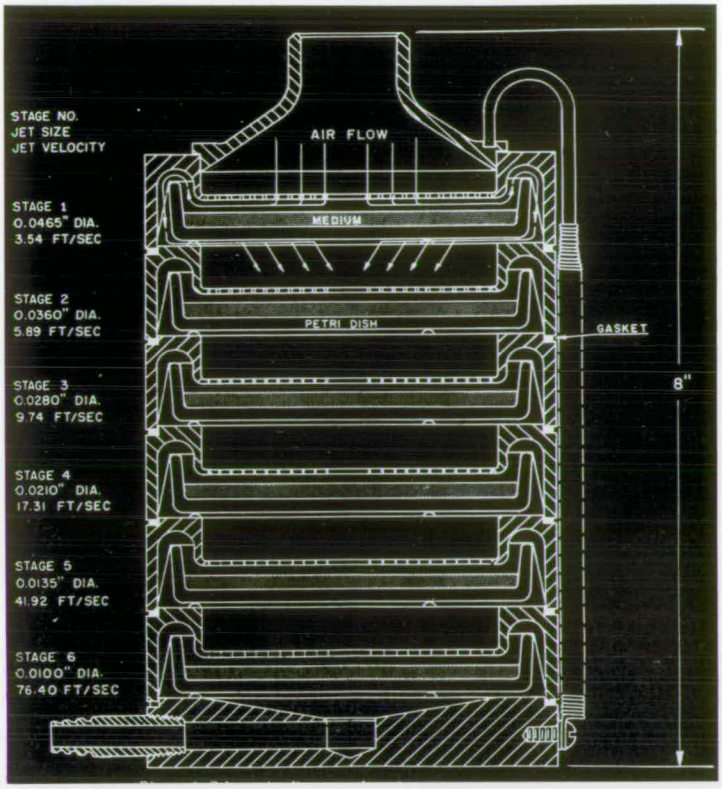


Figure 25:Schematic diagram of a six-stage Andersen sampler

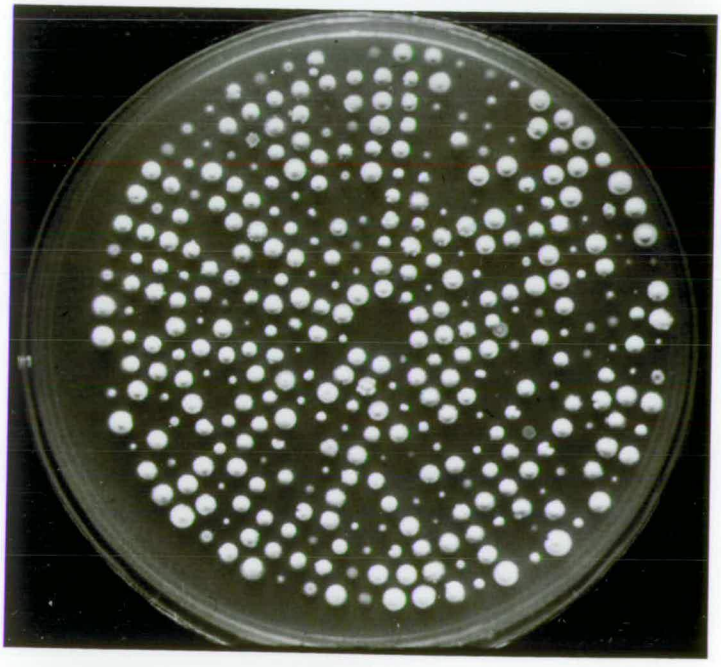


Figure 26:Colonies of mesophilic actinomycetes isolated on malt agar incubated at 26°C demonstrating the pattern of 400 'holes' obtained on stage 6 of an eight stage Andersen sampler(stages numbered 0-7)

APPENDIX C.

Coca's Solution

5g. Na Cl

2.75 g. Na HCO_3

4 g. Phenol

1 l. distilled water

This solution was left overnight before use.

Veronal buffer for immunoelectrophoresis (I.E.) and immunoelectroosmophoresis (I.E.O.); A

7.92 g. barbitone sodium

11.5 ml. N/1 HCL.

1.0 g. Na N_3 (to prevent fungal or bacterial contamination).

1 l. distilled water

Veronal buffered agar for I.E. and I.E.O.

15 g. Ionagar No. 2 (Oxoid)

500 ml. buffer solution A.

500 ml. distilled water.

1% Osmium Tetroxide in Sodium Cacodylate buffer pH 7.4

100 ml. 0.1 M sodium cacodylate

5.4 ml. 0.1 N HCL

94.6 ml distilled water

Osmium tetroxide added to give a 1% solution.

APPENDIX D.

The following books and papers were used for identification of fungi and actinomycetes.

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Wilson, J.W. and Plunkett, G.A. (1965): The Fungous Diseases of Man. Berkley and Los Angeles: The University of California Press.

2) Actinomycetes

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Shirling, E.B. and Gottlieb, D. (1968) Cooperative description of type cultures of Streptomyces II: Int. J. Syst. Bact., 18, 69.

Shirling, E.B. and Gottlieb, D., (1968) Cooperative description of type cultures of Streptomyces III: Int. J. Syst. Bact., 18, 279.

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APPENDIX E.

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Prevalence of farmer's lung in Scotland:a pilot survey.

Grant, I.W.B., Blyth, W., Wardrop, V.E., Gordon, R.M., Pearson, J.C.G and Mair, A.

revalence of Farmer's Lung in Scotland: A Pilot Survey

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ish Medical Journal, 1972, 1, 530-534

mmary

a survey of the farming population of Orkney, Ayre, and East Lothian the prevalence of farmer's lung s estimated at 86 per 1,000 in both Orkney and Ayre and 23 per 1,000 in East Lothian. If cases with a gative farmer's lung hay (F.L.H.) precipitin test are cluded these figures are reduced to 43, 36, and nil spectively, but those for Orkney and Ayrshire are still out 20 times higher than any figure previously reported the prevalence of farmer's lung in Britain.

Regional variations in prevalence are probably related th to climatic conditions and to differences in agriltural methods, the latter often being dictated by onomic circumstances. Nevertheless the prevalence farmer's lung could be reduced considerably by the ergetic application of preventive measures, backed financial incentives. The most important of these are efficient drying of hay and cereals before storage, more extensive use of silage, better ventilation of farm buildings, and the introduction of mechanical feeding systems. Individual farmworkers could be taught how to recognize the early symptoms of the disease and encouraged to wear respirators when handling mouldy fodder.

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Introduction

The disease now called farmer's lung has apparently been known to farmers for over 100 years. Bjornsson (1960) mentioned that his grandfather, an Icelandic farmer born in 1855, suffered from it as a young man, and referred to the disease as "heymaedi" meaning "hay shortness of breath." It was, however, not recognized as a clinical entity until Campbell (1932) published his original account of "acute symptoms following work with hay" affecting farmworkers in Westmorland. The 1931 hay crop had been harvested under unusually wet conditions, and had become very mouldy during storage. When the last part of the stored crop was being fed to cattle between April and June 1932 several farmworkers developed severe dyspnoea, cyanosis, slight dry cough, and mild pyrexia. They were found to have diffuse dry rales and sibilant rhonchi on auscultation and "fine granular stippling" throughout both lung fields on *x*-ray examination.

The term "farmer's lung" was first used by Pickles (1944) when he reported a similar case in Yorkshire, but the nature of the disease remained obscure until Pepys *et al.* (1962) found that precipitins against extracts of mouldy hay ("F.L.H. antigens") were present in sera from patients with farmer's lung. Pepys *et al.* (1963) identified *Thermopolyspora polyspora* (now renamed *Micropolyspora faeni*) as the main source of F.L.H. antigen, and Williams (1963) showed that the inhalation of either F.L.H. antigen or an extract of *M. faeni* reproduced the symp-

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he differential diagnosis from bronchial asthma presented no blems, because for the purpose of the survey a complaint of eze and/or the finding of rhonchi on auscultation automati- excluded a diagnosis of farmer's lung. A few patients who lled the diagnostic criteria also had symptoms consistent with nic bronchitis, and a diagnosis of farmer's lung was ac- ed in these cases only if it was confirmed on critical reas- ment that they satisfied the criteria laid down in 2 (*c*) above.

ults

Orkney all farms were visited on the two selected islands and peration was complete. However, a few subjects (12 in nsay) were not interviewed because of their temporary nce from the islands for reasons unconnected with the stigation. In Ayrshire co-operation was obtained at 91% of ns, and in East Lothian at 87% of farms. In terms of subjects, of the expected number were interviewed in Ayrshire and in East Lothian. The percentage was lower in Ayrshire use the number of persons per farm in the large farms was ller than the average figure used in the calculation of the ected number.

The prevalence of symptomatic farmer's lung was 86 per 00 in both Orkney and Ayrshire and 23 per 1,000 in East

E II—*Prevalence of Farmer's Lung in Orkney, Ayrshire, and East Lothian*

	No. of Farmworkers Interviewed	No. of Cases	Crude Prevalence (per 1,000)	Prevalence Corrected for Sampling Method (per 1,000)
ey (Stronsay and nday)	359	31	86	86*
hire	148	13	88	86 (S.E. ± 19)
Lothian	148	4	27	23 (S.E. ± 13)

ndard error not applicable since no sampling was involved.

hian (Table II). The standard error of prevalence is a meas- of variability due to the sampling method adopted for shire and East Lothian. With prevalences of the order found, errors are inevitably large with samples of the size used in pilot study. Because of these large errors the estimates of the valence of farmer's lung in Ayrshire and East Lothian can be approximate. In East Lothian the overall prevalence ld be as low as 5 per 1,000 or as high as 65 per 1,000. In shire the rate could be as low as 50 per 1,000 or as high as per 1,000. Although there is a slight overlap in these two bable ranges, it can still be said that there is a significant erence, at the 1% level, between the prevalence of farmer's g in the two counties. Since the figure recorded for Orkney the same as the Ayrshire figure, it would be reasonable to ume that farmer's lung was significantly more common in h these counties than in East Lothian, though a direct istical comparison between Orkney and the other two nties could not in fact be made. In the Orkney survey

LE III—*Prevalence of Farmer's Lung in Orkney Farmworkers*

	No. Interviewed			No. of Cases			Prevalence (per 1,000)		
	M.	F.	Total	M.	F.	Total	M.	F.	Total
nsay ..	97	38	135	16	1	17	165	26	126
day ..	137	87	224	11	3	14	80	34	63
otal ..	234	125	359	27	4	31	115	32	86

ble III) there was a higher prevalence in the island of onsay (126 per 1,000) than in the island of Sanday (63 per 00), and this difference was statistically significant ($P = 0{\cdot}05$).

An analysis of the sex distribution of the 48 cases in the three areas (Table IV) showed, as would be expected, that males were considerably in the majority. The most striking feature of the age distribution (Table V) was a considerable excess of elderly

TABLE IV—*Sex Distribution of Cases*

	Male	Female	Total
Orkney	27	4	31
Ayrshire	11	2	13
East Lothian	3	1	4
Total	41	7	48

TABLE V—*Age Distribution of Cases*

	20—	40—	60—	Total
Orkney	8	12	11	31
Ayrshire	5	7	1	13
East Lothian	2	0	2	4
Total	15	19	14	48

patients with farmer's lung in Orkney, but this may merely reflect the age pattern of the farming population in that area.

The symptoms most frequently recorded were dry cough and exertional dyspnoea, which were present by definition in all cases. Episodes of fever were reported in 30%, chiefly in the more severe cases. About 35% of the total farming population in all three areas complained of what they called "irritation" of the throat or nose *during* exposure to dust from mouldy hay, straw, or grain. This symptom subsided an hour or so after exposure ceased and was unrelated to the prevalence of farmer's lung. Radiological abnormalities (Table VI) were observed in

TABLE VI—*Radiological Examination of Lungs*

	Orkney	Ayrshire	East Lothian	Total
No. *x*-rayed	26	12	4	42
Micronodular shadowing ..	2	1	0	3
No abnormality	24	11	4	*39
No. not *x*-rayed	5	1	0	6

*Seven of this group (five in Orkney and two in Ayrshire) had micronodular shadowing when *x*-rayed previously.

only 3 of the 42 patients who were *x*-rayed. In seven others, however, micronodular shadowing typical of allergic alveolitis had been noted previously, during a more acute phase of the illness, and this had presumably cleared before the patients were *x*-rayed in the course of the survey.

Disturbances of pulmonary function (Table VII) were classified as "restrictive" when the FVC was more than 0·5 l. below the predicted normal value, and "obstructive" if the FEV_1/FVC ratio was less than 65%. On this basis a restrictive defect was present in 8 of the 43 patients for whom data were available, an obstructive defect in 12, and a combined restrictive and obstructive defect in three. Pulmonary function tests were normal, according to the criteria adopted, in the remaining 20 patients. A study of the smoking habits of the 43 patients showed that 16 (38%) smoked 10 g or more of tobacco per day,

TABLE VII—*Pulmonary Function—Type of Defect*

	Orkney	Ayrshire	East Lothian	Total
Restrictive	5	2	1	8
Obstructive	10	2	0	12
Restrictive + obstructive ..	2	1	0	3
None	9	8	3	20
Data not available	5	0	0	5
Total	31	13	4	48

s and bronchial asthma, we believe that the prevalence recorded in the survey were broadly accurate within the ts imposed by necessarily crude diagnostic criteria. This ression has received some support from D. Parratt (personal munication, 1971), who tested sera from 25 of our farm-kers by a fluorescent antibody technique. He obtained a tive result in 16 out of 18 patients with symptomatic ier's lung, and a negative result in six out of seven farm-kers in whom this diagnosis was rejected.

hese results suggest that accuracy in the serological diag-s of farmer's lung could perhaps be improved by the use of technique to supplement the standard F.L.H. precipitin If, however, we accept the traditional view that the diag-s of farmer's lung is valid only when the F.L.H. precipitin is positive, the revised prevalence rates for Orkney (43 per 0) and Ayrshire (36 per 1,000) are still more than 20 times ier than any figure previously reported in Britain. On this ence there can be little doubt that the prevalence of farmer's has so far been seriously underestimated. Because of onal differences, the actual size of the problem in Scotland whole is difficult to measure, but on the basis even of the er prevalence rates we have calculated for Orkney and Ayr-e there must be no fewer than 275 cases in these two coun-alone.

Other information obtained from the survey has enabled us to ntify a number of factors which may influence the prevalence armer's lung, and to assess their importance. These can be mmarized as follows:

NFALL

the three areas included in the survey the prevalence of ier's lung was directly related to the rainfall recorded during previous summer, but the difference between the prevalence farmer's lung in Ayrshire and East Lothian was greater than ht have been expected from a comparison of the summer fall figures. This would suggest either that meteorological data er than rainfall (humidity, for example) would have been more vant or that factors not directly related to climate at least tly accounted for the differences in prevalence between these counties.

ICULTURAL PRACTICE

st of the East Lothian farmworkers in the survey were em-yed on large arable farms, and few of them were regularly aged in the feeding of stock. In Ayrshire, on the other hand, st of the farmworkers were employed on dairy farms, and the ding of stock was one of their regular tasks during the winter nths. This was also the case in the small mixed farms and fts in Orkney. These differences in agricultural practice were doubt partly responsible for the higher prevalence of farmer's g in Ayrshire and Orkney than in East Lothian.

The agricultural industry in East Lothian is prosperous, and ny of the farmers there can afford the expensive mechanical ipment required for the rapid drying of hay and cereal crops, h in the field and in storage. Since hay and grain with a low isture content (below 20%) provide an unfavourable medium the growth of fungi and actinomycetes, the economic advan-es enjoyed by East Lothian farmers may have contributed siderably to the low prevalence of farmer's lung in that county. e smaller farms in Ayrshire are less favourably placed in this pect, and in Orkney the situation is a great deal worse, since ny of the small farms possess no mechanical equipment, such balers and binders, which may have to be borrowed from re fortunate neighbours. In these circumstances the farmers may ve no option but to bale their hay and harvest their oats or ley at a time when the moisture content is undesirably high. consequence, the hay bales and corn stacks often become mouldy during storage, and give rise to a serious spore hazard when they are handled inside a farm building during the winter.

Conclusion

Although the pilot survey reported above was conducted on a relatively modest scale, it has shown beyond reasonable doubt that farmer's lung is an important occupational disease in certain areas of Scotland. It is clear, however, even from our limited experience, that much can be done to reduce the prevalence of this disease, or even to eliminate it completely. The measures which in our view can and should be taken to achieve this objective are as follows: (1) the promotion of an educational campaign to inform farmers and farmworkers of the nature of farmer's lung, and of what can be done to prevent it; (2) selective financial assistance for small farms, enabling the farmers to purchase equipment for the rapid and efficient drying of hay and cereal crops; (3) official propaganda, possibly backed by financial incentives, to encourage the use of silage, which carries an insignificant spore hazard, in preference to hay for the feeding of stock in small, ill-equipped farms; (4) encouragement of the provision of well-ventilated stock buildings and of mechanical systems for the handling and feeding of fodder, so as to minimize the dust hazard to which stockmen may be exposed; and (5) personal protection of farmworkers by efficient and comfortable respirators, which have been shown by Gourley and Braidwood (1971) to prevent a recurrence of symptoms in men with a history of farmer's lung when they are re-exposed to a heavy concentration of spores from mouldy hay; to be effective, these respirators must be worn continuously by all farmworkers whenever they are handling mouldy hay, straw, or grain.

We should like to express our thanks to our present colleagues in the Working Group on Fungal Infection and Allergy for helpful advice, and in particular to Dr. Mary Noble and Mr. J. C. Brownlie, of the Department of Agriculture and Fisheries for Scotland, who were two of the most active members of the Working Group until they retired in January 1971. We owe a special debt of gratitude to Dr. J. T. Boyd, Dr. J. W. N. Duerden, Dr. L. A. Hardie, and Dr. A. P. Littlewood, who carried out all the clinical investigations in Ayrshire and East Lothian, and to Mr. A. Inglis, who provided valuable support in the Orkney survey. We gratefully acknowledge the secretarial help we received from Miss Barbara MacMillan. Our thanks are also due to the farmers, their wives, and the farmworkers in the three areas for their wholehearted co-operation.

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ABSTRACT OF THESIS

Name of Candidate Violet Elizabeth Wardrop

Address Department of Botany, King's Buildings, Mayfield Road, Edinburgh.

Degree Ph.D. *Date* January 1974

Title of Thesis Studies on the microflora of farm environments associated with farmer's lung disease

A study of the microflora of various types of farm buildings and products was made in Ayrshire and East Lothian and also of farmproducts in Orkney, with respect to the prevalance of farmer's lung disease in these three regions of Scotland. It was established that there was a correlation between the prevalance of farmer's lung and the state of preservation of farm products such as hay and grain, measured by the numbers of thermophilic organisms isolated both from the farm products and farm buildings. A lower state of preservation of materials in Orkney and Ayrshire, compared with East Lothian correlated with disease prevalences of 86, 86 and 23 per 1000 of the farming population, respectively. The hazards implicated in causing disease symptoms were hay, straw and grain.

Air-sampling of one dairy byre in the East of Scotland was carried out and it was established that high concentrations of propagules of fungi and actinomycetes were recovered from such a building, particularly after unbaling of hay. Figures of up to 7.31×10^6 fungal propagules per cubic metre of air were recorded by using the Burkard volumetric spore trap after unbaling of hay. Air-sampling by the Andersen Sampler was more accurate in quantitative and qualitative terms than that by either the Burkard volumetric spore trap or the gravity Petri-dish technique in isolating fungi and actinomycetes.

Air-sampling of farm buildings on Ayrshire dairy farms by using the Andersen Sampler established that there was, in general, a very high concentration of organisms in the air of such buildings after unbaling of hay in dairy byres and bruising of grain in bruising-sheds. There was a correlation between high numbers of thermophilic organisms, particularly the actinomycete, Micropolyspora faeni, both from the air of farm buildings and from products used in these buildings and the presence of farmer's lung disease on the dairy farms studied. In general, farms with no evidence of farmer's lung disease in the six years previous to the study realised considerably lower numbers of colonies of M. faeni both from farm buildings and from materials such as hay, grain and dust.

Only 43% of sera from individuals with clinical symptoms of farmer's lung were positive to standard farmer's lung hay (F.L.H.) antigen in the initial survey of Ayrshire, Orkney and East Lothian. Antigenic extracts of material from Ayrshire dairy farms and from fungi and actinomycetes isolated from these farm buildings and products were tested against sera from personnel on the farms surveyed. Extracts of hay and grain from farmer's lung disease (FLD) farms were more reactive than those from non-FLD farms but did not correlate directly with reactivity to standard F.L.H. antigen. Fifty-two percent of personnel from FLD farms were serologically positive to F.L.H. or when tested against extracts of M. faeni and 54% of personnel from non-FLD farms were positive to such extracts, but of the former group, 67% were also symptomatic. There was a similar pattern of sensitisation to F.L.H. and to M. faeni on both FLD and non-FLD farms which did not correlate with disease

Use other side if necessary.